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FOOD CHOICES FOR HUNGRY BROILER BREEDERS: DO THEY PREFER QUANTITATIVE OR QUALITATIVE DIETARY RESTRICTION?

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DECLARATION:

I, Louise Anne Buckley, declare that this thesis is entirely my own work, and that it has not been submitted for any other degree or professional qualification. The work is substantially my own and, where more than one researcher worked on the experiment, the contribution of that researcher is outlined in relevant experiment preamble. Any other support provided is outlined in the acknowledgements section of the relevant chapter.

Signed:

Louise Anne Buckley

DEDICATION:

This thesis is dedicated to all the many animals (both past and present) that have I have had the pleasure of spending my life with. They have always brought a smile to my face and rocked my world however crazy and downright quirky they (and I!) might be …

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Contents

Abstract

This programme of research uses choice test methodologies to quantify hungry broiler breeder chickens' preferences for qualitative or quantitative dietary restriction. It begins with an outline of quantitative dietary restriction, its severity and welfare implications before discussing methods of qualitative feed restriction and the difficulties ascertaining whether it represents a welfare improvement.

Chapter two reviews the factors affecting diet preferences and discusses implications for feed restricted broiler breeder diet preferences. Chapter three outlines the use of a closed economy T-maze task to quantity the diet preferences of feed restricted broiler breeders. It concludes that broiler breeders can learn a food versus no food task but find it very difficult to learn a task in which both of the options are rewarded with food and this impeded diet preference quantification. Chapter four demonstrates that severity of feed restriction underlies these difficulties in learning.

In Chapter five, a conditioned place preference task to identify the effects of diets on affective state (hunger versus satiety) is reported. A method validation group demonstrated that broilers show a state dependent preference for an environment associated with *ad libitum* access to food. However, birds failed to show a preference between an environment associated with quantitative dietary restriction and one associated with qualitative dietary restriction. Chapter six applies state- dependent learning (SDL) to quantifying the satiating effects of quantitative and qualitative dietary restriction. However, a validation group suggested that SDL preferences were probably an artefact of the test rather than a genuine state-led preference.

Finally, the overall conclusion that no evidence was found that broiler breeders want, or that their welfare is improved by, qualitative feed restriction was drawn. However, the conditions under which a preference was reliably observed and the presence of hunger – state dependent effects on learning and expression of learnt preferences complicates the interpretation of any findings. Recommendations for further research are highlighted.

1. Feed restriction, hunger and the broiler breeder

1.1. Introduction

Fast growing broilers (chickens reared commercially for meat) have been selected for rapid growth (Weeks, 2004). Broiler breeders (the parent stock) have to be feed restricted to prevent morbidity and mortality and optimise fertility (Hocking *et al.*, 1987; Robinson and Wilson, 1996; Hocking, 2004). This is traditionally done through the feeding of a restricted quantity of high quality feed. Quantitative feed restriction is widely accepted to result in chronic feelings of hunger in the commercial fast-growing broiler breeder (Mench, 2002; de Jong, *et al.*, 2003). Hunger is recognised as an unpleasant sensation (Dawkins, 1990). Therefore, there is a need to address this welfare issue.

With 6.3 million broiler breeders in the UK alone (DEFRA, 2011) the impact of feed restriction on broiler breeder welfare is a considerable one as it effectively results in a very large number of birds chronically suffering. Further, with the worldwide continued increase in the popularity of poultry as a meat source (Weeks, 2004) the number of birds experiencing this form of suffering can be expected to continue to rise in the absence of any intervention. Also, for several years, the age at which most commercially reared broilers are slaughtered has decreased by 1 day per year (Weeks, 2004). Although this trend cannot decrease indefinitely it can be seen that any increase in the *ad libitum* feed intake of broilers is presumably likely to be associated with an increase in the mismatch between actual intake and desired intake in the parent stock. Commercially, slower growing broiler breeder strains (that experience much less or no feed restriction) are unlikely to gain wide acceptance due to increased production costs (De Jong and Guemene, 2011). Therefore, the problem of feeding to reduce hunger whilst preventing obesity is unlikely to disappear in the foreseeable future.

An alternative approach to this conundrum is alter the quality of the feed by reducing the energy density of the feed ration (e.g. Zuidhof *et al.*, 1995; Savory *et al.*, 1996; Savory and Lariviere, 2000; Hocking *et al.*, 2004; de Jong *et al.*, 2005; Sandilands *et al.*, 2005, 2006). Alternatively, this has been achieved by the addition of compounds known to suppress feed intake (e.g. Savory *et al*., 1996; Rozenboim *et al.*, 1999; Savory and Lariviere, 2000; Sandilands *et al.*, 2005, 2006). Finally, some authors use a combination of these approaches (e.g. Tolkamp *et al.*, 2005; Sandilands *et al.*, 2005, 2006). However, these approaches to

hunger management in the broiler are inconclusive with regards to their ability to improve welfare (primarily by improving satiety) (D'Eath *et al.*, 2009). D'Eath *et al.* (2009) suggest that the application of choice test methodologies may be a novel and potentially rewarding way to determine the welfare effects (or otherwise) of feeding a qualitatively restricted diet. Dawkins (2003, 2004) suggests that an animal's welfare can be evaluated by asking whether the animal is healthy and if it has what it wants. Thus, when evaluating the relative welfare of broiler breeders when fed either quantitative or qualitative feed restriction there are two questions to ask: 1) what dietary regime promotes the better physical health status?, and 2) what dietary regime do the birds prefer? Sandilands *et al.* (2005) demonstrated that it is possible to feed a broiler breeder *ad libitum* on a qualitatively restricted diet and maintain good physical health and production similar to that of birds fed on a quantitatively restricted diet. Therefore, the key issue that remains is: do broiler breeders *want* a diet that is qualitatively restricted or one that is quantitatively restricted?

The development of this idea was the starting point for this thesis. D'Eath *et al.* (2009) point out that it seems obvious that animals offered an *ad libitum* choice between high energy density food and low energy density diets when tested in an open economy environment will show a preference for the high energy density diet. For example, Haskell *et al.* (2001) found *ad libitum* fed broilers taught to run down a runway to receive a high energy density food reward gradually run slower when this food reward was switched to a low energy density food reward. However, it is not so clear whether the birds will prefer the high energy density diet when the volume / weight of the diet is much less than the low energy density diet (matched to ensure the same rate of growth irrespective of ration consumed) and no other food is available outside of the test situation (D'Eath *et al*., 2009).

The internal state of the animal is likely to alter the way that it 'evaluates' the various qualities of each diet (Kyriazakis *et al*., 1999) and this may affect the outcome of the decision-making process. Here (in this thesis) the animal is being asked to choose, whilst in a state of chronic feed restriction, between two restricted quantity rations that may have very differing effects on the subsequent feeling of hunger. This is a very different context from a choice in which an animal (± feed restricted during training and testing) is asked to choose between two diets offered on an *ad libitum* basis. It is not clear how broiler breeders (or any animals) will assimilate proximal cues related to satiety when, in a closed economy

environment, they are offered diets that are iso-growth and fed long term under conditions of chronic caloric restriction.

In this thesis it is assumed that if qualitative dietary restriction results in an improvement in the subjective state of the broiler breeder by lessening the feeling of hunger then the birds will prefer it a two-way choice test (or other preference test paradigm) to quantitative dietary restriction. Animals can self-medicate against various nutritional deficiencies (nutrient specific hungers) by showing a state-dependent preference for feed containing high levels of the nutrient in question (chickens and selenium, Zuberbuehler *et al*., 2002; methionine, Steinruck *et al.*, 1990 cited by Forbes, 2007; and calcium, Woodgush and Kare, 1966; Joshua and Mueller, 1979). Further, broilers preferentially select a diet containing an analgesic when lame whereas non-lame broilers do not (Danbury *et al*., 2002) which demonstrates that broilers can use dietary measures to self-medicate against the negative subjective experience of pain. By consuming food, individuals consistently act to self-medicate against another negative feeling – that of hunger – so is it reasonable to assume that broilers will self-medicate against hunger by preferring a diet that results in greater alleviation of hunger?

The remainder of this introduction to the thesis will focus on broiler breeders and the effects of quantitative and qualitative dietary restriction on broiler breeder welfare. It will begin by outlining the effects of quantitative feed restriction on broiler breeder hunger and will discuss whether the broiler is actually capable of feeling 'full'. Qualitative dietary restriction will then be introduced, with special reference to the use of calcium propionate and fibre in the attempt to reduce hunger. Difficulties with interpreting the findings of studies that compare broiler breeder welfare when fed quantitative or qualitative feed restriction will be highlighted and discussed. Finally, the remainder of this thesis will be outlined.

1.2. Broiler breeders, hunger and quantitative dietary restriction

1.2.1. Hunger and satiety: definitions

Before, progressing any further it is useful to understand the terms that are commonly used in literature that examine the effect of feed on indices of hunger and satiety. Hunger has been defined as the negative subjective state of the animal that is chronically undernourished (D'Eath *et al*., 2009). This can, and should be extended, to include acute states of hunger for completeness of definition. It is recognised that mild acute hunger states are not a welfare issue (Dawkins, 1990) with the normal increase in feed motivation that

arises acting to stimulate an animal to engage in appetitive feeding behaviour. However, poor welfare arises from an on-going chronic inability to consummate this appetite.

Satiety is defined as "the feeling of fullness and disappearance of appetite after a meal" (Weber *et al*., 2007) and satiation as the processes that lead to the short term termination of eating. Satiety is considered to be the opposite of hunger (de Graaf *et al.*, 2004) and, as such, a positive affective state. An added complication is that the way in which the satiating (fullness promoting) effects of diets are measured is either through indices of satiation or satiety. 'Satiation' is assessed through the quantity consumed or how quickly an animal stops eating (the less the amount consumed or the quicker the animal stops eating the more satiating the diet is presumed to be). 'Satiety' is assessed through the period of time from cessation of eating to the point at which the animal begins to eat again (the more satiating the first meal is the longer the period of time before the animal starts to consume the second meal). In humans, it is possible to ask the human being to rate their feelings of hunger or satiety. However, this is not an option in animals and thus there is a dependency on using behavioural measurements of feed intake and the time between finishing a meal and starting another one. However, these are externally derived definitions that may or may not be accurate reflection of how full the animal *feels*. An animal may stop eating or delay starting a new meal for many other reasons other than because it feels full. For example, it may stop eating because the food is tainted in some way or because of risk of predation. A second complication is that authors rarely define what they mean by the term satiating, thus complicating any review of the pertinent literature.

In this thesis, unless otherwise stated in the relevant section, all references to hunger refer explicitly to a specific negative affective state in which the animal is motivated to consume food. By contrast, satiety or satiated refers explicitly to a positive affective state in which the animal 'feels full' rather than to the processes leading to the end of meal consumption (although it is accepted that there may be some overlap with the processes of satiation and the affective state of feeling full). Where there is some ambiguity in the papers reviewed (i.e. where the paper uses definitions of satiety and satiation that are measured through feeding behaviour) then 'meal ending' (or a similar phrase) will be substituted for 'processes of satiation' and 'duration until the next meal' (or a similar phrase) will be substituted for 'satiety'.

1.2.2. Severity of quantitative feed restriction

Commercially, broiler breeders are quantitatively feed restricted. Personal observations suggest that feed restriction starts at between 10 – 14 days and becomes increasingly severe over the period of growth. By 6 weeks of age, quantitatively feed restricted broiler breeders are consuming their daily feed ration within about 5 – 7 minutes (Savory and Maros, 1993) and their motivation to feed is as strong straight after consuming this ration as before (Savory, *et al.*, 1993). Motivation to work for feed is positively related to degree of food restriction (Bokkers, *et al*., 2004; De Jong, *et al*., 2003). Using operant technology, Savory *et al.* (1993) concluded that the motivation of chronically feed restricted (to commercial levels) 8 – 20 week old broiler breeders was 3.6 times as strong as broiler breeders fed *ad libitum* and then deprived of food for 72 hours before testing. This highlights that chronic feed restriction is perceived as more aversive than acute feed restriction.

Hocking, *et al*. (1997) compared the total feed intake and feeding rate of 11 week old broiler breeders and a conventional laying breed and found that broiler breeders both consumed more feed in each 105 minute observational period (48g and 18g respectively) and ate more quickly (1.78g/min and 0.83g/min respectively). The Ross 308 Broiler Performance Objectives Manual (Aviagen, 2007) suggests that, under optimal conditions (including *ad libitum* feeding), the average daily feed intake for a 35 day old broiler chick (shortly before slaughter under normal commercial conditions) should be 183g (with an average body weight of 2021g). By comparison, to restrict female (males are slightly heavier) parent stock of this strain to 660g at 35 days (the target weight for a female broiler breeder chick at this age) it is recommended that the birds be restricted to just 44g/day of conventional broiler breeder feed at this age (Aviagen, 2007). Thus, 35 day old broiler breeders are fed just 24% of the expected *ad libitum* intake of broilers of the same age. In both cases a literal interpretation should be avoided as the broilers are a lot bigger by this age and are kept with a much longer light period which promotes feed intake. However, whilst a reduction in light hours will reduce the growth rate of *ad libitum* fed birds, Sandilands, *et al*. (2006) found that, even with only 8 hours light a day, the average weight of 35 day old birds was circa 1100g (feed intake values were not obtained). Thus, it is clear that *ad libitum* feed intake is still far greater than that which conventional feed restriction permits. Correcting for weight, feed restriction is severest around 12 weeks of age at an estimated 33% - 45% of *ad libitum* intake (Hocking, 1993; Savory and Maros, 1993).

1.2.3. Effects of quantitative feed restriction on indices of wellbeing

Although these severe levels of feed restriction are successful in avoiding the health and welfare implications of rapid unchecked growth, the frustration of thwarted feeding motivation and hunger are thought to cause considerable stress to the birds (De Jong, *et al.,* 2002). Behavioural observations indicate that conventionally restricted birds are more active than *ad libitum* fed birds (Savory and Maros, 1993; Hocking *et al.,* 1996, 2001, 2002, 2004) and sometimes demonstrate more aggressive behaviour (Mench, 1988). They also exhibit more drinker - , wall - , and litter-directed behaviour (Savory and Maros, 1993; de Jong *et al.,* 2003; Hocking, *et al.,* 2005) which is thought to be redirected foraging behaviour as a consequence of the thwarted motivational need to forage (Savory and Kostal, 2006). These behaviours can be stereotypic in nature (Savory and Mann, 1997). It is thought that the performance of these behaviours can have de-arousing properties (Hocking, *et al.,* 2005) that allow the bird to cope more successfully with the frustration of being hungry. Feed restricted broiler breeders also show less comfort behaviour such as preening and dust-bathing (de Jong *et al.,* 2003; Puterflam *et al.,* 2006; but see Merlet *et al.,* 2005).

Physiological comparisons between *ad libitum* fed birds and those fed a restricted quantity of feed found a significant positive association between the level of restriction and corticosterone levels (De Jong, *et al.,* 2003; Hocking, *et al*., 2001). However, a meta-analysis by Kyriazakis and Tolkamp (2011) indicates that age and bodyweight are confounded and that, when controlling for this, corticosterone levels are not a useful marker of stress in feed restricted broiler breeders. Other physiological parameters thought to be affected by feed restriction include variation in levels of markers of immune function (Hocking, *et al.,* 2001; but see Hocking *et al.,* 1996, 2001) with increases in the heterophil/lymphocyte ratio of feed restricted birds (Maxwell *et al.,* 1992; Hocking *et al.,* 1993; Savory *et al.,* 1996; but see: de Jong *et al.,* 2003) and elevated glucose/NEFA ratios (De Jong, *et al.,* 2003) observed in feed restricted birds relative to *ad libitum* fed controls. Therefore, combining both the physiological and behavioural findings, it is clear that the welfare of broiler breeders is compromised by conventional feed restriction regimes.

What is clear is that the birds are not simply over-eating for 'something to do' or indulging in redirected behaviour to cope with a stressful environment. Resource cues may potentially increase an animal's motivation to obtain that resource (Warburton and Mason, 2003). However, where food is concerned, it is not a case of out of sight out of mind. When food is

not available birds still behave as though they are hungry. De Jong *et al.* (2005) kept the daily ration constant and changed the mode of feeding either by scattering the feed or by increasing the daily number of feeds to two per day. They found no effect of either treatment on plasma corticosterone levels or compensatory feed intake measurement. This suggests that different methods of feed presentation, whilst keeping feed levels/quality constant, have no effect on hunger levels.

1.3. Broiler breeders and the attainment of satiety

Do broiler breeders actually have the capacity to feel satiated? This is an important consideration as, if they cannot, then it is likely that no dietary modification will result in the alleviation of hunger in the broiler breeder. Broilers consume a quantity of feed that approaches the physical limits of the gastro-intestinal tract (Nir *et al.*, 1978). This suggests that their desired intake, even on a high energy density diet, is already close to being constrained by physical characteristics (Emmans 1997; Illius *et al.*, 2002). Burkhurt (1983) suggests that broiler breeders have disruptions to the 'satiety centre' within their hypothalamus. They found that slow growing chickens with hypothalamic lesions show increased growth relative to slow-growing controls; whereas hypothalamic lesions do not have any effect in fast growing breeds. However, broiler breeders have the capacity to increase both short and long term feed intake to accommodate changes in feed quality or feed regime (reviewed by Nielsen, 2004) and defend energy intake. Fast-growing broilers fed *ad libitum* show similar feeding patterns to slower-growing lines (Howie *et al*., 2009) although they eat faster and have fewer, but larger, meals. A disrupted satiety mechanism (i.e. the birds never feeling full) is presumed to lead to the replacement of normal meal patterns with continuous 'topping up' of gastrointestinal feed contents (Howie *et al.*, 2009). As this was not observed, this suggests that the birds do attain satiety even if only for a short window of time.

However, motivation to eat *can* be affected by alterations to hypothalamic function. Rats with hypothalamic lesions also show increased meal size rather than increased meal frequency with meal size dependent on diet palatability (Sclafani and Berner, 1976). The authors suggest rats with hypothalamic lesions have increased motivation to eat rather than increased hunger or decreased satiety levels. This change in feed intake is thought to be driven by increased sensitivity to the palatability of the diet due to hypothalamic lesions preventing or attenuating the ability of rats to monitor energy homeostasis. Broilers respond to increased fat (though to be highly palatable to lots of species) by increasing their feed

intake (Forbes, 2007). However, decreased palatability is associated with a decrease in feed intake (Sclafani and Berner, 1976). Despite these findings, an interpretation of broiler breeder feeding motivation along the same lines as the conclusions drawn in the Sclafani and Berner (1976) study would probably be erroneous. Feed restricted broiler breeders consume more than 200% of their daily water requirements (Burnett and Buckley, unpublished undergraduate dissertation), with polydipsia thought to be due to a redirected attempt to alleviate hunger (Kostal *et al.*, 1992; Hocking *et al.*, 1996). It is unlikely that the hedonic qualities of water stimulate over consumption. Therefore, a more reasonable explanation is that it is an attempt to alleviate hunger or a frustrated foraging motivation. Thus, if 'normal' chickens have the capacity to experience the subjective state of satiety and show similar feeding patterns, then it should be inferred that broiler breeders also have this capacity.

Studies that reared broiler breeders to different levels of feed restriction report that there is a positive relationship between severity of feed restriction and strength of motivation to access a food reward (Savory *et al*., 1993; Bokkers *et al*., 2004). This highlights that broiler breeders are also sensitive to the degree of feed restriction. If the strength of the motivation reflects the magnitude of an animal's underlying affective state (Dawkins, 1990) then it should be assumed that broiler breeders can feel more or less hungry with the aversiveness of the hunger experience correlated with the severity of the feed restriction. Qualitatively restricted diets are an attempt to ameliorate, at least partly, the unpleasant state that is hunger.

1.4. Qualitative feed restriction: a more welfare – friendly approach to feeding?

1.4.1. What is qualitative feed restriction?

Qualitative feed restriction can be defined as the restriction of caloric intake by adjusting the quality of the feed such that the animal voluntarily restricts its energy intake to below that of animals fed *ad libitum* on a high energy density diet. This may be achieved by reducing the energy density of the diet or by the inclusion of compounds in the feed ration that reduce intake of the high energy density diet. Some researchers (e.g. Zuidhof *et al.*, 1995; Kapowska *et al.*, 2005) combine quantitative and qualitative feed restriction by offering a fixed quantity of a quality – adjusted diet that increases time taken to consume the ration but is still less than what would be consumed if the bird was given *ad libitum* access to this feed ration. For the purpose of this thesis all feeding regimes in which the diet is quality – adjusted with a view to

reducing energy consumption or increasing the time taken to consume the daily feed ration will be defined as qualitative feed restriction.

1.4.2. Compounds used to reduce feed intake in broiler breeders

1.4.2.1. Introduction

Numerous compounds have been trialled for possible inclusion in the diets of broiler breeders to try to suppress their appetites. These include compounds designed to primarily increase gastric satiety (through increased volume, delaying gut emptying, etc) whilst reducing the energy density of the diet (although some compounds are also thought to also exert some post-absorption effects on satiety through volatile fatty acid production). Examples of compounds used to significantly dilute the energy density of diets include sugar beet or potato pulp (Savory *et al.,* 1996; Hocking *et al.*, 2004; Enting *et al.*, 2007; Nielsen *et al.,* 2011), sawdust (Savory *et al.,* 1996) and oat hulls (Zuidhof *et al.,* 1995; Sandilands *et al.*, 2005, 2006; Hocking *et al.,* 2004; Hocking, 2006; Enting *et al.,* 2007; Nielsen *et al.,* 2011). Other compounds are primarily expected to be absorbed through the gastrointestinal tract and thought to exert their action metabolically, presumably by acting on pathways associated with hunger and satiety. For example, flenfluramine (Hocking and Bernard, 1993; Rozenboim *et al*., 1999; Kapowska *et al.*, 2005), jojoba meal (Vermaut *et al.,* 2005), and propionate (as one of its various salts, usually calcium) (Savory *et al.,* 1996; Kapowska *et al.,* 2005; Sandilands *et al.,* 2005, 2006) have all been used experimentally to decrease appetite and reduce energy intake. Finally, some authors have combined compounds: for example, oat hulls and calcium propionate (Sandilands *et al.,* 2005, 2006; Tolkamp *et al.,* 2005). The most successful diet to date in terms of managing growth rate whilst allowing the birds to consume a ration *ad libitum* has been the Sandilands *et al.* (2005) diet that combined calcium propionate and oat hulls within the feed ration. Therefore, it is this diet and its appetite suppressing components that will be focussed on in the next three sections.

1.4.2.2. Calcium propionate

Propionate is a food additive with anti-fungal and anti-microbial properties (Al Lahham *et al.,* 2010). As one of the naturally occurring volatile fatty acids (VFAs), propionate is present in some foods such as cheese and milk (Al Lahham *et al.*, 2010). It is a key end product of microbial fermentation in the gastrointestinal tract (predominantly the caecum of monogastric species) of poultry (Annison *et al*., 1968) and other species. However, its

inclusion in feed is associated with a depression in feed intake in chickens (Pinchasov and Jensen, 1989; Pinchasov and Elmaliah, 1994, 1995; Sandilands *et al.*, 2006), pigs (Giesting and Easter, 1985; Castell *et al.*, 1994) and an increase in reported satiety in humans (Liljeberg *et al.*, 1995). This reduction in feed intake occurs even if propionate is crop-fed (i.e. bypasses sensory-led oral preferences) (Pinchasov and Jensen, 1989) indicating that its mode of action is not predominantly related to palatability or other similar issues.

High levels of VFAs are associated with metabolic acidosis which depresses the appetite (Owens *et al.*, 1998) and ruminant animals will limit feed intake to prevent production of excess VFAs by ruminal bacteria (Provenza *et al.*, 1994). However, acidosis does not appear to underpin its effects on feed intake in monogastric species. Neutralising the acid does not affect intake (Pinchasov and Jensen, 1989). Therefore, propionate is thought to act primarily through its effects on satiety by affecting various physiological pathways and processes (Arora *et al.*, 2011). Propionate is associated with delayed gastric emptying in humans (Darwiche *et al.*, 2001) and stimulates the adipose tissue - related satiety hormone Leptin (Al-Lahham *et al.*, 2010). Altered post consumption insulin and glucose levels (both compounds the levels of which in the blood are associated with the control and stimulation of feed intake) have also been observed (Liljeberg *et al.*, 1995, 1996). However, when controlling for palatability, propionate was found to have no effects on indices of satiety in humans fed a sodium propionate-enriched breakfast (Darzi, *et al.*, 2008); although the inclusion rate was much lower than that used experimentally with broiler breeders.

Oral consumption of propionate has been associated with nausea (in humans) (Frost *et al.*, 2003) and gingivitis (inflammation of the gums) (Al Lahham *et al.*, 2003). Tolkamp *et al.* (2005) combined calcium propionate (inclusion rate: 6 – 9%, increased with age) with a mash diet and were forced to prematurely cull the treatment group at 12 weeks of age (33 weeks earlier than the intended end date) after the development of oral lesions. However, this may also be caused by the fineness of the mash (see: Gentle 1986) that could be avoided by pelleting the diet (Tolkamp *et al.*, 2005). The authors did not observe lesions in similarly aged broiler breeders fed propionate at a higher inclusion rate (9 – 10%, increased with age) in a previous experiment (Sandilands *et al.*, 2006). However, calcium propionate has also been implicated in mild crop erosion (Bolton and Dewar, 1994). Although this represented one affected bird out of a sample size of four the authors point out that this had never been observed before in hundreds of routine avian examinations at the centre (birds fed a diet not

containing propionate). This introduces the possibility that calcium propionate may reduce feed intake either by an increase in satiety (a positive effective state) or by its aversive properties such as taste or oral irritation (associated with negative effective states) or by a combination of both factors. It should be noted that both outcomes would cause a decrease in feed intake but only one would be as a consequence of satiety. The valence of the affective state if both positive and negative effects are present is unclear and likely to reflect the relative weighting that the bird gives to each of the physiological signals received.

1.4.2.3. Fibre

Fibre is primarily (but not exclusively) considered a low or non-nutritive bulky filler of diets with variable effects on satiety dependent on the type of fibre under consideration (Slavin and Green, 2007) and its viscosity (Kristensen and Jensen, 2011). Fermentable (soluble) fibres may also increase satiety through the metabolic effects of volatile fatty acids produced during bacterial fermentation (Enting *et al.*, 2007*;* Bosch *et al.*, 2009). Oat hulls are insoluble and resistant to fermentation in the gut (Stephen *et al.*, 1997). However, both fibres have been used to modify appetite in animals (Zuidhof *et al.*, 1995; Savory *et al.*, 1996; Jewel *et al.*, 2000; Savory and Lariviere, 2000; Hocking *et al.*, 2004; de Jong *et al.*, 2005; Sandilands *et al.*, 2006; Weber *et al.*, 2007), although high levels of fermentable fibres are associated with behavioural indicators of ill-health in broiler breeders (Nielsen *et al.*, 2011). This suggests that non-fermentable fibres may be more useful than fermentable fibres (but see Hocking *et al.*, 2004).

Studies looking at feed intake and satiety in the short term suggest that it is the weight or volume of the food that determines both the process of satiation / meal ending (i.e. individuals consume less calories during meals with a low energy density) (see: de Graaf *et al.*, 2004) and the feeling of satiety post-meal. This may be combined with release of the cholecystokinin gastric hormone and with a delay in the rate of gastric emptying (Weber *et al.*, 2007) and increased water retention (e.g. Hocking *et al.*, 2004). Fibre has been associated with improvements in satiety in the very short term (measured both subsequent motivation to feed in animals (e.g. Robert *et al.*, 1997; Savory and Lariviere, 2000) and humans (e.g. Burley *et al.*, 1993; Samara and Anderson, 2007), and also in some qualitative subjective ratings of satiety by humans (e.g. Burley *et al.*, 1997; Isaksson *et al.*, 2009; Lyly *et al.*, 2009; Wanders *et al.*, 2011; but see Blundell and Burley, 1987).

The longer term effects of high fibre diets on satiety are less clear. Firstly, both dieting (Geliebter *et al.,* 1996) and long term ingestion of bulky diets (JoRgensen *et al.,* 1996) result in alterations to the size of the gastrointestinal tract. This is likely to alter the efficacy of a given volume of food to stimulate gastric stretch receptors. Further, dependent on the type, fibre can be digested and utilised by the animal to various degrees (see Johnston *et al*., 2003) by a process of fermentation. This microbial process yields VFAs (propionate being the second most common VFA found in the avian caecum, Annison *et al*., 1968) which can be used as an energy source. Although the contribution of fibre to energy is slight there is some evidence that the avian digestive tract can adapt to being fed even fibre with a low level of digestibility over time and use it as a food source (see Johnston *et al.,* 2003). It is possible that this long term adaptation in the utility of fibre as an energy source may result in modifications to the relative preference for high fibre food. This may be in the direction of a relative decrease in preference for the fibre enhanced diet that may be observed if the diet becomes less satiating as the gastrointestinal tract adjusts to a more bulky ration. Alternatively, an increased relative preference for the fibre enhanced diet as the bird gets better at digesting the fibrous content may be observed. This may be confounded by real changes in relative growth supported by each diet as the animal gets better at extracting energy from fibre (see a review by D'Eath *et al.,* 2009). Thus, it is necessary to demonstrate that this is not the case when assessing relative diet preferences in diets purporting to support similar rates of growth (Nb. similar concerns also apply in the use of calcium propionate).

Butterwick and Markwell (1997) found no effect of fibre on satiety in feed restricted dogs (45% of *ad libitum* intake) fed iso-caloric diets when an energy value of 8.36kj / g was assigned to the fibrous component of the diet (and estimated to contribute less than 3% of energy intake). The authors note that this approach was problematic in that it may have under or over-estimated the contribution of fibre to metabolised energy. However, when Tolkamp *et al*. (2005) calculated the basal feed intake (i.e. the basic diet to which oat hulls and calcium propionate were added) they found no difference between the birds fed *ad libitum* on the qualitatively restricted diet and birds fed the quantitatively restricted diet option. Neither was there a significant difference between the birds in bodyweight at the three reported stages (6, 12 and 18 weeks). Thus, it seems reasonable to assume that neither calcium propionate nor oat hulls provide an appreciable amount of additional metabolisable energy in the quantities offered. This may be due to a reduction in the digestibility coefficient. Enting *et al.* (2007) found that when broilers are fed on a quantity restricted diet (± a variety of dietary dilutions

that reduced energy and nutrient density by 12 – 23%) digestibility coefficients and metabolisable energy were significantly lower when energy density was reduced.

Finally, fibrous substances may also be associated with aversive compounds such as tannins, pectins and alkaloids that may also depress intake (Guillemet *et al.,* 2007) and lead to their avoidance in a two way choice test with low fibre diets. There is some evidence that increased gut distension can be aversive (Bardos, 2001; Bardos *et al.*, 2002) which may be a problem when feeding a bulky diet. However, high levels of fibre are not automatically associated with a decrease in palatability: in a two way ad libitum access for 15 minutes choice test, Weber *et al*. (2007) found that the diet preferences of dogs were more sensitive to protein level than fibre level. However, as it is proposed to keep the protein content identical (per ration offered) in the studies carried out as part of this thesis, the impact of increasing fibre on broiler breeder preferences under conditions of feed restriction remains unclear.

1.4.2.4. Compound synergism and feed intake

Combining calcium propionate and oat hulls in the same feed had a synergistic effect on feed intake with feed intake lower than when either compound was fed separately (Tolkamp *et al.,* 2005). However, it should be noted that their measure of satiety was feed intake. This may or may not be a good proxy of satiety (feeling of fullness) as other factors may depress feed intake (for example, oral pain, or issues associated with diet palatability or bulkiness). Further, it is possible that each compound, whilst working synergistically to reduce intake was not working synergistically to improve affective state. For example, one compound may reduce intake by improving satiety (a positive affective state) whereas another may reduce intake by causing discomfort (e.g. lesions from calcium propionate or gut distension from the high fibre content of the diet). Thus, it seems reasonable to propose that studies investigating the diet preferences of feed restricted broiler breeders should at least consider testing these compounds separately in choice test experiments.

1.4.2.5. Is qualitative feed restriction more welfare friendly than quantitative feed restriction?

It is not clear whether qualitative feed restriction improves indices of wellbeing in the broiler breeder when compared with conventional quantitative feed restriction. Most physiological parameters remain similar between birds fed either quantitative or qualitative dietary restriction: for example, immune response to a challenge (Hocking, 2006) and other

immune system parameters (Savory, *et al.,* 1996; Sandilands *et al.,* 2005, 2006; but see: Zuidhoff, *et al.,* 1995; Hocking, *et al.,* 2004). Where parameters do vary interpreting the findings may be problematic. For example, non-esterified fatty acids (NEFA) are considered to be a biomarker of negative energy balance (Emery *et al.,* 1992). Glucose levels relative to NEFA levels are known to be increased in broiler breeders that are quantitatively feed restricted relative to less restricted birds (de Jong *et al.,* 2003). Whilst the glucose : NEFA ratio is reduced in birds fed a qualitatively restricted diet differences in how quickly a ration is digested (for example) make this parameter problematic to interpret (D'Eath *et al.,* 2009).

Most differences have been observed when using behavioural observations although these are equally difficult to interpret. Feeding patterns are considered to be more normal with the bird more in control of meal initiation and termination (see: D'Eath *et al.,* 2009; Kryiazakis and Tolkamp, 2011). As control is considered to be important to animal welfare (Wiepkema and Koolhaas, 1993), D'Eath *et al.* (2009) argue that qualitative feed restriction may improve welfare by giving broiler breeders control of their feeding patterns. However, in the absence of improved satiety, the welfare improvements of this greater freedom to determine their own feeding patterns may be illusory. Most studies indicate that activity levels are similar between treatment groups (e.g. Zuidhof *et al.,* 1996; Sandilands *et al.,* 2006; but see: Sandilands *et al.,* 2005). The total time budget of behaviours related to foraging (to include feeding behaviours directed at non-food objects) is similar with high fibre diets (Sandilands *et al.,* 2005) but altered with diets containing appetite suppressants (Sandilands *et al.,* 2005, 2006) which suggests that different qualitative diets may have different behavioural effects. This strengthens the proposal that choice tests should test the satiating effects of compounds separately.

Interpretation of the birds' behaviour in relation to the relative satiating effects of the different diets is problematic. For example, a reduction in oral behaviours directed at non-feed objects may be interpreted as a reduction in the motivation to feed or as a reduction in the time available to demonstrate these behaviours (low energy density diets take longer to consume) (D'Eath *et al.,* 2009). Whilst rate of consumption has been used a marker of feeding motivation (i.e. a proxy marker for hunger level) (e.g. Terlouw *et al.,* 1991; Bergeron *et al.,* 2000; Sandilands *et al.,* 2005, 2006), its application to measuring the relative satiating effects of qualitative and quantitative diets is more problematic. A key issue when comparing motivation to eat of different treatment groups is differences in styles of eating developed

prior to testing (in a competitive environment broiler breeders have to eat very quickly when fed quantitatively restricted feed rations) (Sandilands *et al.,* 2005). Further, it is not clear whether birds should be offered the same type of food reared on in the 'rate of eating' test (leading to different diets offered to each treatment group during testing) or whether this should be standardised between groups (leading to both novelty and contrast effects) (D'Eath *et al.,* 2009). Operant tests suggest that birds reared on qualitative feed restriction are less motivated to eat shortly after feeding compared with quantitative dietary restriction but show a similarly high level of responding when tested hours after the last feed (Savory and Lariviere, 2000). This suggests that qualitative feed restriction may confer acute satiating benefits, but that the results are very short lived in chronically feed restricted birds. However, it is possible that different diets result in different points at which sensory-specific satiety (the state in which the individual stops eating but will resume eating if offered different food stuffs) (see Sorensen *et al.,* 2003) occurs and that this is driven by a different state other than a feeling of fullness. Finally, by measuring satiety indirectly through feeding behaviour, the problem arises that 'ending the meal' and 'duration until next meal' may not by synonymous with the animal actually feeling full.

In summary, the combined physiological and behavioural observations do not conclusively indicate whether qualitative feed restriction confers welfare benefits through a reduction in feelings of hunger in the feed restricted bird. Thus, there is a need for additional approaches to investigate this welfare problem. This includes the application of choice tests to determining which system of feed restriction the broiler breeder prefers (as suggested by D'Eath, *et al.,* 2009).

1.5. Outline for the remainder of this thesis

The remainder of this thesis will be concerned with the use of various methods of assessing the preferences that broiler breeders may show for either quantitative or qualitative feeding regimes.

In chapter two, the use of choice tests to determine diet preferences will be outlined including their benefits and drawbacks. This chapter will then primarily focus on factors known to affect diet preference and the potential implications for the direction and underlying aetiology of any preference observed in broiler breeders.

In chapter three, an experiment that looked at the preference of feed restricted broiler breeders for either quantitative feed restriction or qualitative feed restriction is reported. In this study two different types of qualitative feed restriction are used. One diet includes calcium propionate and the other ground oat hulls and the birds are asked to associate different coloured T – maze arms with different dietary outcomes in a closed economy task in which no food was available outside of the test situation.

Chapter four takes a detour away from the primary research question and explores the effect of feed restriction severity upon the ability of the birds to express a rational preference for a large, rather than a small, feed portion. Birds are tested in an experimental set – up similar to that used in chapter three.

In chapter five, the focus then returns to investigating hungry broiler diet preferences using a closed economy conditioned place preference paradigm. The qualitative feed restriction option is a diet with added calcium propionate. The birds are asked to choose between an environment associated with quantitative feed restriction and an environment associated with qualitative feed restriction. It is assumed that the bird will prefer to spend time in the environment it associates with feeling less hungry whilst in.

In the final experiment chapter (chapter six), the use of state – dependent learning to determine the effect of different diets on the feeling of satiety is explored. Three different experiments are reported including diets that have been qualitatively adjusted using either calcium propionate or cellulose. The primary hypothesis explored is that the birds will prefer a food reward received under conditions of high deprivation (i.e. very hungry) over conditions associated with low deprivation (i.e. much less hungry).

Finally, chapter seven summarises the findings of the study and suggests methods for further investigating the effect of qualitative feed restriction on broiler breeder welfare.

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2. A critical review of the key influences on dietary preference

2.1. Introduction

The aim of this review is to explore the issues associated with the presence of diet preferences in animals with particular emphasis on how the issues raised may affect hungry broiler breeder preferences for quantitative or qualitative dietary restriction. It begins with an introduction to choice tests and identifies the key approaches taken to identifying diet preferences in animals before discussing the advantages and disadvantages of these methods. Further relevant issues to diet preference quantification are then discussed. The discussion then turns to actual diet preferences and the factors that influence, or are thought to influence, the diet choices that an animal displays. Finally, potential reasons for any preferences that broiler breeders may exhibit during the proposed choice tests and why it is essential to adopt a multimodal approach to quantifying preference are outlined and discussed.

2.2. What are choice tests?

Simple choice tests are tests in which an animal is given access to two (or more) resources and allowed to choose freely between them (Lawrence and Illius, 1997). Where the resources are substitutable, the chosen option is said to be the preferred resource (Kirkden and Pajor, 2006). Although, as Kirkden and Pajor (2006) point out, sometimes non substitutable resources (i.e. resources that satisfy different motivations) are used in order to identify which resource is most important to the animal. For example, Schutz *et al.* (2008) investigated the importance of shade during the summer to dairy cows. They deprived cows of the ability to lie down for varying periods of time (0, 3 or 12 hours) and then offered them a choice between lying down in an un-shaded area or standing up in a shaded area. This sort of choice test enables researchers to move beyond a simple assessment of preference to investigating the strength of a preference for a given resource. However, before the strength of any preference can be quantified it is necessary to identify whether a preference does, in fact, exist. This is the domain of the simple choice test. As this is often the extent to which diet preferences have been examined in the literature the focus will remain on this rather than on strength of preference.

Motivation, choice and preference tests are well established methods of examining an animal's welfare and can provide a powerful measure of what commodities and animal wants, needs or places a high value upon (Kirkden and Pajor, 2006). Although short term

motivational priorities (Bateson, 2004) or impulsivity (Abeyesinghe *et al*., 2005) may affect choices made on basis of immediate need rather than long term welfare gain (Dawkins, 1990) they are still considered an important way of assessing an animal's subjective wants.

2.3. Choice test methodologies commonly used to assess diet preferences

2.3.1. Two – pan tests

By far the most common method of identifying an animal's diet preference is through the 'two-pan test' (e.g. Mehiel and Bolles, 1984; Steinruck *et al.,* 1990, cited by Forbes, 2007; Killeen *et al.,* 1993; Sclafani *et al.,* 1994; Warwick and Weingarten, 1996; Warwick *et al.,* 1997; Lucas and Sclafani, 1999; Guillemet *et al.,* 2007). This approach involves giving an animal free access to two different diets simultaneously for a fixed period of time. Occasionally, more than two foods are offered simultaneously (e.g. 2 – 4 options, Moon and Zeigler, 1979; 2 – 3 options, Gous and Swatson, 2000) although there is a limit to how many diet choices an animal can compare simultaneously (Raffa *et al.,* 2002). The measure of preference is normally the quantity consumed of each of the diets (or solutions).

'Two pan' tests are normally conducted over a short (e.g. 5 minutes, Guillemet *et al.*, 2007; 30 minutes, Sclafani and Ackroff, 1993) or medium term period of time (e.g. 24 hours, Mullen and Martin, 1992; 2 days, Bellush and Rowland, 1985). Sometimes diet preferences are measured over a much longer period of time. For example, Kutlu and Forbes (1993) offered heat stressed and unstressed chickens the choice between diets supplemented with ascorbic acid or a control diet and measured intake of each over a period of 7 and 14 days. Similarly, Zueberbuehler *et al.,* (2002) assessed selenium deficient layer hen preference for selenium enriched and selenium deficient diets over a period of six weeks. This has the advantage of allowing changes in preference with increased exposure to the two options to be observed. For example, Zueberbuehler *et al.* (2002) found that the hens showed a preference for the selenium enriched diet only during the first 3 weeks of choice testing (which probably reflected the fact that the chickens had corrected their selenium deficit by this point). The disadvantages of the two-pan approach are that limited data is obtained compared with operant tests (Rashotte and Smith, 1984) and other factors may influence preference such as satiety (Araujo and Milgram, 2004). This may be absolute (the animal stops eating any of the options temporarily) or sensory – specific (a declining satisfaction during a meal with the consumption of that food / diet option).

2.3.2. One – pan tests

Alternatively, animals might be given access to each option sequentially, with a period of time (e.g. 24 hours) allowed to elapse between each presentation to ensure that the effects of satiety do not influence intake of either option offered. This is referred to as a one-bottle test (e.g. Sclafani and Ackroff, 1993; Lucas and Sclafani, 1999). Researchers usually combine the one – and two – pan test approaches in the same study (Sclafani and Ackroff, 1993; Lucas and Sclafani, 1999; Warwick and Synowski, 1999). However, Lucas and Sclafani (1999) compared preference for polycose or corn oil solutions. They found that the rats consumed far more corn oil solution than polycose solution in sequential one – pan tests, but drank more polycose solution in the subsequent two – pan test. This highlights that diet preference quantification is problematic.

2.3.3. Operant choice tests

Operant choice tests are tests in which the animal is required to perform an operant response (behaviour) to receive a reward or avoid an unpleasant outcome (Kirkden, 2010) and are widely used to assess preferences for many different resources in a range of species (see Kilgour *et al.,* 1991). The operant response required can involve any behaviour but typical methods require an animal to press a lever, bar or foot pedal or to peck a key. The tasks require that an animal learn to associate a given response with a given outcome (Rashotte and Smith, 1984). For example, Araujo and Milgram (2004) taught beagle dogs to associate three different objects with three different outcomes (diet A, diet B or no food) and dogs then choose between objects rather than between diets. More typically they represent a high tech version of traditional two pan tests in which the animal chooses between two different operant tasks (e.g. red and green pecking keys, Marsh *et al.,* 2004). The operant response then results in access to the food reward (usually for a finite period of time or quantity before a further operant response is required to access further food).

These approaches have the advantage that they do not require the animal to have *ad libitum* access to the diets so multiple intra-animal tests can be performed without the risk of satiety or post-ingestive effects affecting preference when only initial sensory-led preference is of interest (Matthews and Temple, 1979; Rashotte and Smith, 1984; Araujo and Milgram, 2004). It also limits the risk of the animal becoming obese (Araujo and Milgram, 2004) through multiple *ad libitum* access to food during tests. The approach is potentially particularly advantageous when quantifying hungry broiler breeder dietary preferences for quantitative or qualitative dietary restriction as it enables the researcher to ensure that the

animal is making a choice relevant to conditions of feed restriction. In other words, the animal is genuinely asked to choose between performing an action (operant response) that results in a large quantity of low quality food and an action that will result in a much smaller quantity (but iso-energetic) of high quality food (as proposed by D'Eath *et al.*, 2009).

However, these tasks are more complex than simple one and two-pan choice test methodologies and have the disadvantage that the animal must learn the association between a given stimulus, the operant behaviour to be performed and a particular diet type before it can meaningfully express any dietary preference. These complexities mean animals need to be highly trained (Rashotte and Smith, 1984) and the number of training trials needed may be extensive before the criterion for success is reached. Also, some operant tasks can be difficult for a given animal to associate with that particular outcome (Dawkins and Bearsley, 1986). However, the use of naturalistic tasks may improve training outcomes and are to be recommended in preference to tasks that are more abstract (Cooper, 2004) and may reduce the need for extensive training.

The operant method employed can be low tech such as the weighted push door approach adopted by Petherick and Rutter (1990) to assess the motivation of feed restricted hens to access food. Other approaches include the use of Y – mazes (e.g. Phillips and Strogan, 2007). However, computer technology can also be used (e.g. Johnson *et al.*, 1986; Colbert and Rowland, 2005; also see Kagel *et al.*, 1995). The latter has the advantage that it is less prone to inadvertent observer bias (Cooper, 2004). It can also allow a large amount of data to be collected, with multiple trials over a long period of time (Rashotte and Smith, 1984). Further, it is possible to easily vary the work rate (e.g. number of pecks to access a given fixed quantity of resource) which allows the research to move beyond a simple identification of preference and to assess the strength of this preference (Rashotte and Smith, 1984).

However, experiments involving computer-controlled operant equipment have the disadvantage that more complex equipment can be prone to malfunctioning (Cooper, 2004). In general, operant tasks are sometimes dogged with high inter-animal variability in preference (Lawrence and Illius, 1989) with small sample sizes decreasing the likelihood of the experiment detecting a genuine preference (Paterson-Kane *et al.*, 2008). Also, side biases sometimes prove problematic (Rofe and Anderson, 1970). Excluding animals with side biases and increasing sample size (Rofe and Anderson, 1970) can help with identification of the diet preference of the majority. Where diet preferences are being measured over a long time frame (e.g. several weeks or months) in a closed economy apparatus this is likely to be particularly problematic (although the only practical way to undertake this). Equipment cannot easily be

re-used to test multiple animals in a single experiment during a reasonable timeframe in this scenario making this financially costly. Further, automatic feeders that can cope with different diets and ration sizes are not always commercially available (e.g. for delivery of broiler breeder mash or pelleted diets, author's own investigations).

2.3.4. Negative and positive contrast methods

Runways have been employed with mixed success by some researchers to assess the motivation of animals to access food (e.g. Chickens, Petherick *et al.*, 1992; Bokkers *et al.*, 2007). However, this approach can also be combined with negative and positive contrast methods to indirectly assess diet preference. For example, Haskell *et al.* (2001) investigated successive negative contrast in broilers conditioned to expect a high value food reward and suddenly switched to a low value food reward (the chickens gradually ran slower post-switch). Similarly, starlings suddenly switched from meal worms (high value) to turkey crumbs (low value) consume less turkey crumbs post switch than starlings maintained on turkey crumbs throughout the study (Freidin *et al.*, 2009). However, the method utilised may affect whether a contrast effect is observed. Deer exhibit evidence of 'disappointment' when encountering a less liked food in a simultaneous contrast test but fail to show this in a successive negative contrast paradigm utilising the same two diets (Bergvall *et al.*, 2007). Further, Petherick *et al.* (1992) found that acute feed restricted birds ran faster than *ad libitum* fed birds in a food rewarded runway task, but the severity of feed restriction (6, 12 or 18 hours) did not affect runway speed due, it is thought, to the ceiling effect. Therefore, the methodology adopted is critical to the success of this approach (although clearly this applies to other methods of diet preference too). Many of the advantages and disadvantages of operant testing also apply to this approach and, indeed, operant technology is sometimes employed to determine the presence of negative or positive contrast effects (see Flaherty, 1996).

2.4. Other issues, potential problems and limitations when measuring diet preferences

2.4.1. Absolute and partial preferences

A basic, but often erroneous, assumption of diet preferences is that an animal will show an absolute preference for one diet when given a free choice between two different diets (Emmans, 1991). In practice, animals often show partial preferences (e.g. Rutter, 2006) that may be diurnal (Rutter, 2006) or affected by relative novelty of each diet (ideally pre-

exposure should be standardised to overcome this). Previous nutritional history (Forbes, 2007), current nutritional state (e.g. Steinruck *et al.*, 1990, cited by Forbes, 2007; Hughes and Woodgush, 1971) or life stage (Emmans, 1991) of the animal tested may also affect diet preferences. Thus, diet preference may be a dynamic preference as the animals' state and experiences are continuously changing. This means that the absence of preference or existence of partial preferences may be problematic to interpret (Duncan, 2005). Where both diets provide similar levels of all nutrients but vary across some other dimension (e.g. addition of a low or non-nutritive filler or compound with appetite suppressant properties) diets are imperfectly substitutable goods.

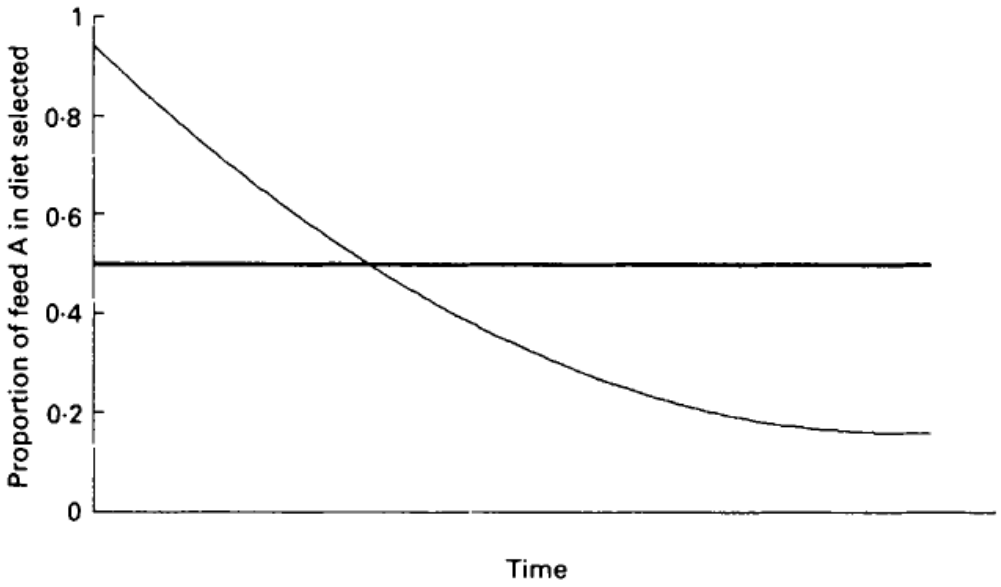
Emmans (1991) argues that any inferences about diet preferences can be improved by looking for evidence of transitive diet choices. Emmans (1991) also stresses that it is impossible to identify whether the bird is showing indifference or nutritional wisdom when the optimal diet mix is 50:50. Therefore, the optimal proportional intake of each should be adjusted well away from this value (Forbes, 2007). However, the impact of this on the interpretation of broiler breeder dietary preferences for quantitative or qualitative dietary restriction is uncertain as both diets are nutritionally complete.

2.4.2. Changing diet preferences

Often, diet preference tests do not last long enough to accommodate the effects of post-ingestive feedback on diet preference (Emmans, 1991). Figure 1 illustrates how an animal's preference for one of two diets may theoretically alter over time as a consequence of post-ingestive feedback. Thus, a robust investigation of diet preference should entail a series of preference tests carried out over a reasonably long time frame.

This has implications for the quantification of feed restricted broiler breeder dietary preferences as the severity of conventional feed restriction varies with the life stage (Savory and Maros, 1993). For example, the bird may prefer a high energy density diet for the first few weeks of feed restriction (when feed restriction is comparatively mild), but be prepared to trade quality for quantity as feed restriction gets increasing more severe.

Figure 1: Theoretical model illustrating how diet preference may alter over time (source: Emmans, 1991)



2.4.3. Assessing preferences in feed – restricted animals

One key issue that deserves attention is the context within which the preference of the broiler breeder for either quantitative or qualitative dietary restriction is trained and tested. An animal's preference is heavily influenced by the context in which assessment takes place (Mendl, 1999; Bateson, 2002; Dawkins, 2003) and a change in context between the test environment and the 'real – life' environment the results are being extrapolated to may mean that the preference is no-longer relevant (see Mason *et al.*, 1997). An animal may choose different diet options under different circumstances (Hutchings *et al.*, 1999). Closed economy experimental set ups (in which the animal can only access the resource, such as food, during testing) are more likely to reveal an animal's true preferences than open economy set ups (see: Dawkins, 1990; Mason *et al.*, 1997). Thus, the broiler breeder should be tested under conditions of feed restriction in a closed economy environment to ensure greater parity with the dietary conditions the bird would experience under conditions of quantitative or qualitative dietary restriction.

Further, an animal may choose a high energy density diet over a low energy density diet when given *ad libitum* access to both during testing as a strategic feeding behaviour to maximise energy intake when this option is available rather than as a genuine aversion to the low energy density diet (Guillemet *et al.*, 2007). Thus, an animal should be offered restricted (ideally iso-energetic) portions of each diet option during testing.

2.4.4. Training animals to associate cues / stimuli with diet quality or quantity

The need to train under conditions of feed restriction and to not allow *ad libitum* access to feed during testing places restrictions on the choice test methodologies that may be utilised. Traditional two pan tests are no-longer appropriate. Therefore, it becomes necessary to use an choice test methodology in which the animal effectively chooses between two conditioned stimuli which are associated with a restricted ration of conventional feed (fed to quantitatively restricted birds) and a restricted ration (that is iso-energetic with the other diet option) of a qualitative-adjusted diet. The animal effectively has to learn two tasks: firstly, to associate each diet option with its respective post-ingestive effects, and secondly, to associate that diet option with a secondary stimulus.

Nutritional history can affect preferences (Forbes, 2007) and birds can show neophobia when encountering a new diet (Haskell *et al.,* 2001). Thus, both diets should be novel to the animal at the start of training. It is essential that chickens are given time to learn about the relative nutritional consequences of eating different diets (Pousaga *et al.,* 2005). However, the time needed to learn about the relative effects of each diet depends on the state of the animal, the contrast between diets and the effects of the diet on the animal's [nutritional or energetic] state (Kyriazakis *et al.,* 1999). Feed restricted rats have been shown to learn a diet preference in as little as four days (Wawick and Synowski, 1999). Further, chicks taught to associate different coloured food with high and low protein composition switch preferences within 3 days when the colour : protein content association is reversed (Hannah, 2001). This suggests that, if the contrast between diet options is large, hungry broiler breeders will rapidly learn an association between the diet options and their respective effects on hunger level.

Whilst animals may learn when diet options are presented simultaneously, learning may be improved by alternating diet options offered (e.g. every day) (Forbes, 2007). This is because, when several foods are consumed in a short time scale, the animal finds it difficult to distinguish between the post-ingestive effects caused by each diet option (Van Wieren, 1996). Duncan and Young (2002) found that herbivores found it difficult to associate each diet with its post-ingestive effects when the diets were presented together. There is no reason to assume that chickens would be any different in this respect. Forbes (2007) suggests that alternating every half day may be sufficient for chickens as hens have a faster metabolism and can detect a change in protein levels within a few hours. Thus, any preference test with broiler breeders should probably separate exposure to each diet for a period of several hours to maximise the ability of the broiler to associate each diet with its relative satiating effects.

With any associative task, testing must be preceded by a period of training in which the animal learns to associate the secondary cues with the primary reinforcers (the diet options). Visual cues, such as the colour of food (e.g. as used by Kutlu and Forbes, 1993), are readily learnt (Wilcoxon *et al.,* 1971; Pousaga *et al.,* 2005), probably because direct sensory cues have a very close association with nutritional value (Forbes and Kyriazakis, 1995) or other aspects of food 'qualia'. By contrast, the location of the food (e.g. within the left or right arm of a T-maze) is a much less powerful cue (Forbes and Kryriazakis, 1995) and, therefore, the animal will probably find it more difficult to learn an association between location and food quality or quantity. However, animals can use more distal cues such as location to learn about food quality although changing positional cues (e.g. switching diets from left to right) will impede the building of associations (Steinruck *et al.,* 1990 cited by Forbes, 2007). However, side biases in chickens are a common problem (Rick D'Eath, personal communication) so positional changes may be advised (with a different cue used to signify diet option availability) to prevent an apparent position preference and / or apparent diet preference being confounded.

2.5. Control of feed intake

Feed intake is closely related to diet preference. Indeed, most choice test methodologies measure relative feed intake in a simultaneous presentation two pan test (e.g. Warwick and Weingarten, 1996; Warwick *et al.,* 1997; Lucas and Sclafani, 1999; Guillemet *et al.,* 2007). Or, alternatively, by measuring relative consumption of each when presented sequentially (e.g. on different days, Sclafani and Ackroff, 1993; Lucas and Sclafani, 1999; Warwick and Synowski, 1999). Thus, it is useful to begin the review of factors influencing diet preference with a discussion of factors known to, or thought to, control or affect food intake.

In the short term, highly palatable diets (usually high energy density) tend to be less satiating and diets with poor palatability (usually low energy density) tend to be more satiating (Drewnowski, 1998). Thus, volume and / or weight of food consumed rather than energy content affects satiation (Bell *et al.,* 2003; de Castro, 2006; Rolls, 2009) with meal size controlled by a combination of gastric distension and release of satiety factors from the gut such as cholecystokinin (Woods *et al.,* 1998). Consequently, how much energy is consumed is directly related to the energy density of the diet (de Castro, 2006). Greater than 40% of short term energy intake is directly attributable to the energy density of the diet (Stubbs and Whybrow, 2004). This still leaves a large percentage of food intake affected by other factors such as palatability. Some theorists (but see Stubbs and Whybrow, 2004)

suggest reducing the energy density of diets is an effective tool in both the prevention and treatment of obesity (for a review see Rolls *et al*., 2005). This is provided that the low energy density diets are perceived to be as (or more) satiating and palatable than high energy density diets (Drewnowki, 1998) as people (and animals) will pick the diets they perceive as more rewarding (Appelhans, 2009). In reality, palatability and low energy density rarely coincide and is a cited reason for failed dieting in humans (Drewnowski, 1998).

However, longer term controls on feed intake may reflect the relative increase in importance of other physiological controls in managing feed intake. For the animal, volume may simply be a proxy measure for energy intake based on eating history and volume preference modified if feedback from energy density changes (Stubbs and Whybrow, 2004). Thus, changing the energy density of a diet may only result in short term deviations from a preferred level of energy intake. Long term, only 7% of variance in energy intake of humans can be attributed to energy density of the diet (Stubbs and Whybrow, 2004) but, in the longer term, the underlying goal of alteration in energy intake is not certain. A popular theory is that animals eat to maximise energy intake relative to constraints (e.g. Emmans and Kyriazakis, 2001). However, Ketelaars and Tolkamp (1992) point out that animals do not always eat to constraints, that physical boundaries (i.e. gut size) are modifiable and that intake is affected by life stage and reproductive status. Further, bodyweight in many adult individuals is remarkably stable over a long period of time (Wilding, 2006) and there is some evidence that people will reduce energy intake after a period of over-eating (Cornier *et al.,* 2004; but see: Cornier *et al.*, 2007).

An alternative viewpoint is that, in the longer term, animals' eat to maintain energy homeostasis, with deviations from a given level of adiposity being detected by changes in levels of biomolecules such as the appetite hormone leptin (de Graaf *et al.,* 2004). Animals often increase volume of feed consumed when diets are diluted to maintain energy intake (e.g. rats, Adolp, 1947, cited by Ackroff, 2002; locusts, Raubenheimer and Simpson, 1993: broilers, Leeson *et al.*, 1996a, 1996b; but see Ackroff, 2002) with multiple metabolic and CNS pathways activated to defend energy homeostasis when animals are feed restricted (Woods *et al*., 1998). This suggests that there is a 'set point' of energy intake that animals eat to consume to maintain a given level of adiposity. Under this theory, animals deprived of their preferred energy intake (i.e. not in a status of energy homeostasis) experience metabolic hunger (see: Stanley *et al.,* 2005; Morton *et al.,* 2006). If broiler breeders are sensitive to energy balance, when fed *ad libitum*, they will consume to meet their perceived energy requirements. If this is so, then a qualitatively restricted diet will not alleviate their metabolic

hunger (e.g. Day *et al.*, 1996; Savory *et al.*, 1996) as they are not moved closer to their homeostatic set point. Thus, preference for high energy density diets may be enhanced or the birds may become indifferent between iso-energetic diets differing in density under conditions of feed restriction.

Voluntary feed intake may also be affected by the external and internal costs and benefits of consuming a given diet (Tolkamp and Ketelaars, 1992; Provenza, 1995; Illius *et al.*, 2002; Woods, 2002).Examples of this include: the presence of dietary toxins (Provenza, 1995), the oxidative stress of metabolising the food (Tolkamp and Ketelaars, 1992; Ketelaars and Tolkamp, 1996) or risks associated with obtaining the food (Illius *et al.*, 2002). Thus, some animal nutritionists argue that the amount of food that the animal chooses to eat varies according to the circumstance as the animal either tries to optimise foraging (Simpson *et al.*, 2004), feed intake (Ketelaars and Tolkamp, 1992a, 1992b; Tolkamp and Ketelaars, 1992; see: Emmans and Kyriazakis, 1995) or to minimise total discomfort (Forbes 2007) rather than as an attempt to attain some fixed energy or nutrient set-point. However, this may conflate the process of satiation (the processes which lead to termination of eating) with satiety (the affective state of feeling 'full') as an animal may terminate a bout of eating before becoming satiated if consuming the diet is associated with unpleasant consequences. In other words, an animal may be prepared to accept a level of hunger to avoid the costs of increased feed intake. This is of particular relevance to researchers quantifying the effects of calcium propionate and fibre on satiation, satiety and diet preference in feed restricted broiler breeders.

2.6. Evolutionary history and diet preference

Animals show dietary preferences which would have improved the fitness of their ancestors (Moss, 1999) and so mechanisms that control these preferences would have been heavily selected for (Yearsley *et al*., 2006). Thus, captive animals are likely to still use cues from previously successful foraging strategies and diet compositions that no-longer benefit them in the captive environment (Illuis *et al.*, 2002). Some taste preferences are thought to be innate (Rogers and Blundell, 1991; Sclafani, 2001). However, post-ingestive feedback enables the hedonic value of the food to the animal to be modified to reflect the value of the food to the animal's survival (Provenza, 1995).

An important corollary to this is that short and long term diet preferences may not be the same. Animals quickly associate the sensory cues from food with their post-ingestion consequences (Sclafani, 2001; Swithers and Davidson, 2008) and this will influence

subsequent diet preference. The ability to modify diet selection on the basis of experience is particularly valuable to omnivores, such as the domestic chicken (Rovee-Collier *et al*., 1982) that obtain nutrition and energy from numerous diverse sources (Rogers and Blundell, 1991). Thus broilers may be quick to modify their diet preferences on the basis of the relative rewarding effects of a quantitative - or qualitative – restricted diet.

However, rapid detection and modification of diet preference may be a double edged sword: rats (another omnivore) quickly increase feed intake to defend a fixed daily intake of energy when fed a diet diluted with cellulose (Adolp, 1947, cited by Ackroff, 2002). By contrast, the domestic cat (carnivorous species generally only consume foods that are similar in energy density) responded to a diet diluted with cellulose by maintaining the same quantity of feed and lost weight (Kanarek 1975, cited by Ackroff 2002). This has potential implications for the value of qualitatively restricted diets for broiler breeder welfare. If they exert their satiating benefits by 'tricking' the animal's physiological system into thinking that the energy or nutrient state is closer to homeostasis than it is, then the chicken may be quicker to detect this satiety 'hoodwinking' faster. This will result in a relatively short time window of benefit for the bird.

It may also necessitate progressive increases in dietary dilution and / or inclusion rate of appetite suppressants to maintain any 'illusion' of homeostasis. This has been shown to be necessary for birds fed *ad libitum* on a qualitatively restricted diet where the goal was voluntary restriction of energy intake to levels close to that achieved with quantitative feed restriction (e.g. Sandilands *et al*., 2005, 2006). Broiler breeders' increase feed intake to defend energy intake when fed a reduced energy density diet (e.g. Leeson *et al.,* 1996a, 1996b). This suggests that broilers are tracking energy (or some other nutrient, as all are likewise diluted). Thus, if the broiler is 'metabolically hungry' a qualitatively restricted diet is likely to have only short term benefits (if any).

Alternatively, it is argued that intensive selection for fast growth has resulted in a degree of decoupling between a broiler's biological needs (i.e. adequate food to maintain a healthy rate of growth) and the associated feeling of hunger that signals that need (Duncan, 2002). However, fast-growing commercial lines of broiler still retain the same patterns of feed intake as slow-growing lines (Howie, *et al.,* 2009) and can select appropriately from diets varying in protein and energy to maximise rate of growth and feed conversion efficiency (Gous and Swatson, 2000). Thus, it is expected that broiler breeder diet preference will still be influenced by their phylogeny. However, how proximal cues are evaluated in the development of diet preferences when both options are iso-growth and fed under conditions

of caloric restriction is unclear. This represents a unique scenario and has not previously been examined in any species (D'Eath, *et al.,* 2009).

2.7. Appetitive aspects of diet preference

"Appetitive behaviour is the variable, flexible, searching phase of a behavioural sequence, indicative of the need or desire to achieve a certain goal" (Zimmerman, 2010, pg. 33). Where consuming food is the goal (or, the consummate act), the behaviour(s) performed to try an acquire food represents the appetitive phase of foraging behaviour. Whilst not strictly a diet preference (and therefore largely outside the remit of this review) a few words remain pertinent as animals may have preferences that relate to *how* they acquire food. The phenomenon of contra-free loading is a classic example. This relates to the observation that sometimes animals show a preference for working for food (either through naturalistic or operant based tasks) over food that is freely available (see Inglis *et al.,* 1997). However, propensity to contra-free loading is reduced by food deprivation (e.g. Bean *et al.,* 1999; Lindqvist *et al.,* 2002) suggesting that state can affect appetitive preferences.

The state of the animal has been shown to affect other appetitive aspects of foraging behaviour as well and the effects of this on the animal's behaviour in a choice test methodology should be considered. For example, when looking at more naturalistic behaviour, Day *et al.* (1999) found that hamsters will preferentially hoard sunflowers (a high fat food) after a period of food deprivation but that the strength of this preference disappears when hamsters are not food deprived.

The operant literature also indicates that state can affect the preferences observed. Food response appears to affect flexibility of response. When given a choice of pressing any lever to receive an identical food reward, hungry rats pick a lever and largely stick with the same one whereas less hungry rats will switch between levers (Carlton, 1962). Animals tend to be risk averse and prefer the operant task that results in a fixed quantity of food delivered after each e.g. lever press (see review by Kalcenik and Bateson, 1996a; Bateson and Kacelnik, 1998) rather than an operant task that results in a variable food delivery. This is despite the mean quantity delivered over the session being identical irrespective of which operant behaviour is performed. However, the reverse preference or indifference between the two options is sometimes observed (Caraco *et al.,* 1980; Young *et al.,* 1990, and others; but see review by Bateson and Kacelnik, 1998) when the animal is severely feed restricted during training and testing.

Finally, the state of the animal during training of an operant response can affect the value that the animal places on performing a particular task. Starlings were taught to peck either a red or green pecking key to obtain an identical food reward. The birds were trained with one colour key when food deprived (high deprivation) and the other key when not food deprived (low deprivation). When later tested for evidence of an operant preference, birds showed a preference for pressing the coloured key associated with high deprivation over the coloured key associated with low deprivation (Marsh *et al.,* 2004). Similar results have been obtained in a range of species (fish, Aw *et al.,* 2009; locusts, Pompilio *et al.,* 2006; pigeons, Gipson *et al.,* 2009, rats, Kurtz and Jarka, 1968; and starlings, Marsh *et al.,* 2004) and using a variety of choice test methodologies (Pecking keys, Marsh *et al.,* 2004; Y – maze, Aw *et al.,* 2009; food paired with an odour, Pompilio *et al.,* 2006).

2.8. Sensory – led aspects of diet preference

Animals can show a preference for a particular diet based on sensory-led aspects of the food. Chickens are primarily visually orientated with respect to food recognition (Gentle, 1985). They often show an initial preference based on colour of the food (although this can be confounded with olfactory cues, Chagneau *et al.,* 2006). For example, chickens prefer light coloured foods to darker colour foods (Chagneau *et al.,* 2006). Where food has been artificially stained, chicks show a preference for red crumbs over green or black if the crumbs are presented on the pen floor (Roper and Marples, 1997). Interestingly, the authors found converse results when the food was placed in a petri dish. This indicates that even the manner in which feed is supplied can influence the direction of any preference observed (see also Roper, 1990).

Broilers show an age - dependent preference for pellets of a particular size (Portella *et al.,* 1988) with older birds preferring larger pellets than younger birds. Texture of the pellet also influences preference, with soft pellets consumed more slowly by broilers than identically sized hard pellets (Picard *et al.,* 1997, cited by Bouvarel *et al.,* 2009) although size, colour and texture of pellets are often confounded in studies (Bouvarel *et al.,* 2009) making inferences more problematic. However, energy content of the pellets can over-ride pellet texture (hard versus soft) in broilers (Bouvarel *et al.,* 2009). Further, pairing an unpleasant post-ingestive effect with the initially preferred coloured mash results in chicks switching preference to the alternative coloured mash (Capretta, 1961) although some colours are easier to associate with unpleasant consequences than others (Rowe and Skelhorn, 2005). This indicates that nutritional factors can modify any sensory - led preferences. However, it should

be accounted for in any preference test with broiler breeders as, whilst mash diets are useful in the initial stages of preference determination, the contrast between sensory aspects is likely to be greater than when the diets are pelleted.

2.9. Palatability and diet preference

Palatability is defined as "the momentary subjective orosensory pleasantness of food" (Stubbs and Whybrow, 2004) and its exact nature is still unclear (Yeomans *et al.*, 2004). It has been suggested that the concept of palatability is defined in a circular way as being synonymous with feed intake (Tolkamp, personal communication). However, whilst to all practical intentions this is necessarily the case when investigating diet preferences in animals, the human literature is able to bypass this issue. This is achieved by the use of subjective ratings scales (or similar) that ask the human to rate how attractive they find a given food (even if they don't want to eat it now). The concept of 'liking' versus 'wanting' is important distinction to make as humans may not always consume the food they find most hedonically rewarding (see: Mela, 2006).

Need has been shown to increase preference for foods containing the nutrient the animal is deficient in (e.g. calcium hunger in layer chickens, Woodgush and Kare, 1966; Joshua and Mueller, 1979). However, other theorists propose that some foods may be rewarding independent of the animal's state of need (i.e. independent of under-nutrition or mal-nutrition or short term hunger states) (e.g. Mela, 2006; Epstein *et al.*, 2003). Access to highly palatable foods increases reported appetite at the start of a meal (Yeomans, 1998), rate of intake (Johnson *et al.*, 1986; Ramonet *et al.*, 1999) and short term intake of food during a meal (see review by Sorensen *et al.*, 2003). Self-reported levels of hunger increase during the early stages of a meal when the food available is rated as highly palatable by the participants (Yeomans 1996). Savory and Mann (1999) found that broiler breeders showed stereotypical pecking post consumption that increased with meal size which suggested that the birds were experiencing an increase in feeding motivation in the earlier part of a meal. Thus, providing feed restricted broiler breeders with a high energy density diet that is expected to be perceived as highly palatable by the birds may exacerbate frustration by lack of adequate provision of rations.

Palatability is thought to be modifiable by post-ingestive feedback (Forbes, 2007). Thus the relative apparent palatability in animals may differ according the amount of exposure an animal has to a particular diet. However, the hedonic value of food and its ability to act as a reinforcer are not always considered equivalent (e.g. Epstein, *et al.*, 2003, 2007) with an

individual able to like a food without necessarily wanting it (Mela, 2006) at a particular state (i.e. when satiated).

Some researchers (e.g. Matthews and Temple, 1979; Araujo and Milgram, 2004) extol the value of their specific choice test methodologies in avoiding the complications of post-ingestive feedback in the determination of diet preferences. These include methods such as operant and second-order conditioning in which the animal receives a very small food reward repeatedly (e.g. Matthews and Temple, 1979) or learns to associate secondary cues with specific food rewards and then chooses between the secondary cues in the choice test (e.g. Araujo and Milgram, 2004). The rationale for this is that this methodology ensures that they measure palatability without other factors such as post-ingestive effects and satiety affecting preference. This seems a peculiar rationale. The avoidance of post-ingestion derived preferences may be erroneous when trying to quantify the preferences an animal may exhibit over the longer term. After all, the researchers are trying to determine preferences that the animal will 'naturally exhibit'.

Food that is initially very unpalatable, such as pig food containing bitrex (Blair and Fitsimmons, 1979, cited by Forbes, 2007), can become completely 'palatable' within a few trials if there are no unpleasant consequences (or the good outweighs the bad). Likewise, diets that are initially palatable can quickly become unpalatable if paired with an unpleasant post-ingestive consequence such as the nausea-causing drug lithium (Burritt and Provenza, 1989). Therefore, it is expected that very hungry animals (such as broiler breeders) will be quick to learn about the relative hunger-reducing benefits of two different diet regimes.

However, palatability driven diet preferences can be modified by post-ingestive feedback in two different ways. Firstly, they can be modified by a change in hedonic status associated with the nutrient. This can be a relative shift in preference for a given diet relative to the same diet before post-ingestive feedback or relative to another diet (after experiencing the post ingestion effects of both diets). An example of the former would be that lambs in a state of metabolic acidosis consume a solution containing bicarbonate (which neutralises the acid) but prefer water to this solution in a two pan test (Phy and Provenza, 1994). Similarly, chickens prefer calcium-rich foods when calcium deficient but not when in calcium homeostasis (Woodgush and Kare, 1966; Joshua and Moeller, 1979) and humans show less disgust towards unpalatable food when food deprived (Hoefling *et al.*, 2009). Secondly, post-ingestive feedback may modify a preference for a specific diet relative to a another diet (such that the animal now prefers diet A to B) or the flavours associated with these diets or nutrients (Mehile, 1991, cited by Provenza, 1995; Sclafani, 1991).

Thus, broiler breeders may show a preference for a quality-adjusted diet under conditions of feed restriction because they find it more palatable than conventional broiler breeder feed when in a state of hunger (due to post-ingestive feedback). Alternatively, they may show a preference because, although the relative sensory pleasure of the low energy density diet remains less, under the current conditions there is a hedonic shift towards preferring a more satiating diet (if it is). In contrast, the birds may show a preference for the high energy density diet if there is no hedonic shift and if birds prioritise short term hedonic, sensory-led gain over a longer term feeling of satiety (assuming qualitative diets do confer greater satiety).

2.10. Energy and diet preference

One prediction regarding diet preference is that the more a nutrient is needed the more it should shape an animal's diet preference. As energy is required in a large amount it is expected to have a strong influence (though not always) (Provenza, 1995). Drewnowski (1998) suggests that energy dense diets are preferred over low energy density diets because they are usually more palatable. Alternatively, or as a causal agent for animals finding high energy density diets more palatable, high energy density diets enable the animal to consume its energy needs more quickly, leaving more time available to meet its other needs (e.g. predator avoidance, mating, territory defence, etc).

Rats develop flavour preferences based on their association with calories and prefer a flavour associated with energy over one that is not, but show no preference between flavours when the nutrient and caloric load are held constant. When the energy density of one solution was increased this was preferred (Mehiel and Bolles, 1984). Animals (including broilers, Bouvarel *et al.,* 2009) usually show a preference for high energy density diets over low energy density diets (e.g. Bolles *et al.,* 1981; Laska, 2000; Laska, 2003; Guillemet *et al.,* 2007). However, the highest energy density diets are not automatically the most preferred in a two way choice test (Warwick and Weingarten 1996; Warwick *et al.,* 1997; Lucas *et al.,* 2007; Ackroff, 2008) suggesting there may be a preferred level of energy density. Evidence suggests this varies with diet history (Meiselman *et al.,* 1974, cited by Stubbs and Whybrow, 2004), age and sex (see review by Drewnowski, 1998) and state of deprivation (e.g. Day *et al.,* 1999; see Warwick and Synowski, 1999). Thus, this preference may be a dynamic rather than a static preference. This further emphasises the importance of quantifying diet preference over a longer timeframe than is often the case.

If animals have to balance their intake of lots of different nutrients independently in order to optimise their intake to meet their nutritional and energetic requirements (and there is growing evidence that they do, see: Simpson *et al*., 2004) then it makes sense to not eat a very energy dense diet. This is because, by doing so, the animal may enter either a state of malnourishment (inadequate nutritional status; adequate energy intake) or a state of obesity (adequate nutrients, too much energy).

Ad libitum fed rats were given a two – bottle choice test between high (1.6kcal/g) and low concentrations (0.2kcal/g) of various macro – nutrient solutions (same nutrient, different concentration for the individual rat). Rats preferred a low concentration to a high concentration of glucose, fructose, sucrose and maltodextrin, but showed a tendency to prefer the high concentration when the test solutions contained corn oil. Further testing showed that when a stronger concentration (3.2 kcal/g) was compared to a 0.2kcal/g solution rats preferred the low concentration of corn oil (Warwick *et al.,* 1997). Similar results were reported by Warwick and Weingarten (1996) and Lucas *et al*. (1997). These findings are thought to be due to highly satiating solutions (more concentrated solutions and / or simple sugar based solutions compared to starch or fat) having limited ability to act as a reinforcer due to rapid satiation / temporary cessation of eating (Lucas *et al.,* 1997).

This preference for diets that are not too energy dense also exists when multiple nutrient diets of varying density are compared (see a review by Ackroff, 2008). Rats given a choice between iso – energetic solutions in which the absolute number of calories varied during the conditioning trials (through adjustments in volume) only showed a tendency to prefer the solution associated with the greater total number of calories (Mehiel and Bolles, 1988). These findings suggest that rats' dietary preferences are more sensitive to the energy density of the diets consumed than the volume (or total number of calories provided) of diets consumed.

However, these studies utilised solutions rather than diets and it is not clear whether these preferences would remain under more 'normal' conditions. It would be useful to repeat the work of Mehiel and Bolles (1988) using rats (or, indeed, broiler breeders) to assess the effects of feed deprivation on how an animal weights the energy density and volume (total amount of energy) of food received when forming preferences. Further, most studies allow *ad libitum* access to conditioning solutions during one bottle 'association' training (Capretta, 1961; Mehiel and Bolles, 1984; Bellush and Rowland, 1985; Mullen and Martin, 1992; Sclafani and Ackroff, 1993; Lucas and Sclafani, 1999; Yinn *et al.,* 2005). The more tightly controlled studies couple intake of one solution or diet to the other (to ensure equal intake during conditioning trials) on the basis of energy and volume (Taylor, 1977; Warwick and

Synowski, 1999; Kimura *et al.*, 2003), energy only (Davis *et al.*, 2007) or volume only (variable energy density) (Mehiel and Bolles, 1984, 1988). It would be useful to see more studies (like Davis *et al.*, 2007, but closed economy) in which volumes are restricted (i.e. fully consumable during the training) but varied between options whilst equalising absolute caloric intake between options during conditioning trials. This would be closer to the choice between qualitative and quantitative feed restriction that broiler breeders would be asked to make.

2.11. Macronutrients and diet preference

Preferences for nutrients go beyond a simple energy-led preference. Otherwise, animals might be expected to be indifferent to iso-energetic diets, whereas evidence shows that this is not the case. Animals develop preferences for flavours associated with specific macronutrients when offered a choice between different macronutrients presented in iso – energetic solutions (Ackroff, 2008). Fat is a weak reinforcer of flavour – nutrient conditioning that sometimes requires conditioning enhancement with the addition of sweeteners (Ackroff, 2008) to reliably condition a preference. Thus, high carbohydrate solutions are often preferred when offered as pure nutrient solutions (Ackroff, 2008). However, when infused as a mixed nutrient, high energy density solution high fat solutions are preferred to iso – energetic carbohydrate rich alternatives (Ackroff and Sclafani, 2006). However, again, it is not clear whether this preference may be modified over the longer term. As Ackroff (2008) notes, some nutrients are harder to condition a flavour, suggesting that the animals find it harder to associate some nutrients with outcomes than others. It may be necessary to run conditioning trials over a longer time frame to ensure that associative strength is identical between diets.

2.12. Quantity versus quality and diet preference

The key question posed in the current thesis is whether feed restricted animals prefer a large quantity of low energy density food or a small volume of high energy density food. There is a lack of studies investigating this in the existing literature. Further, few studies specifically investigate any preferences of animals for a high fibre diet or a low fibre diet. Feed restricted sows preferred concentrated (high energy density) diets over a high fibre (low energy density) diet (Guillemet *et al.*, 2007). However, this study was designed to test sensory – led diet preferences and the sows were not given time to learn about the post – ingestive effects of each diet. Further, testing took place in an open economy (fed outside the test situation) environment with *ad libitum* access to both feeds during the choice test (a simple two – pan

design). Consequently, as the authors note, the preferences observed may have reflected a feeding strategy rather than a genuine aversion to the high fibre diet.

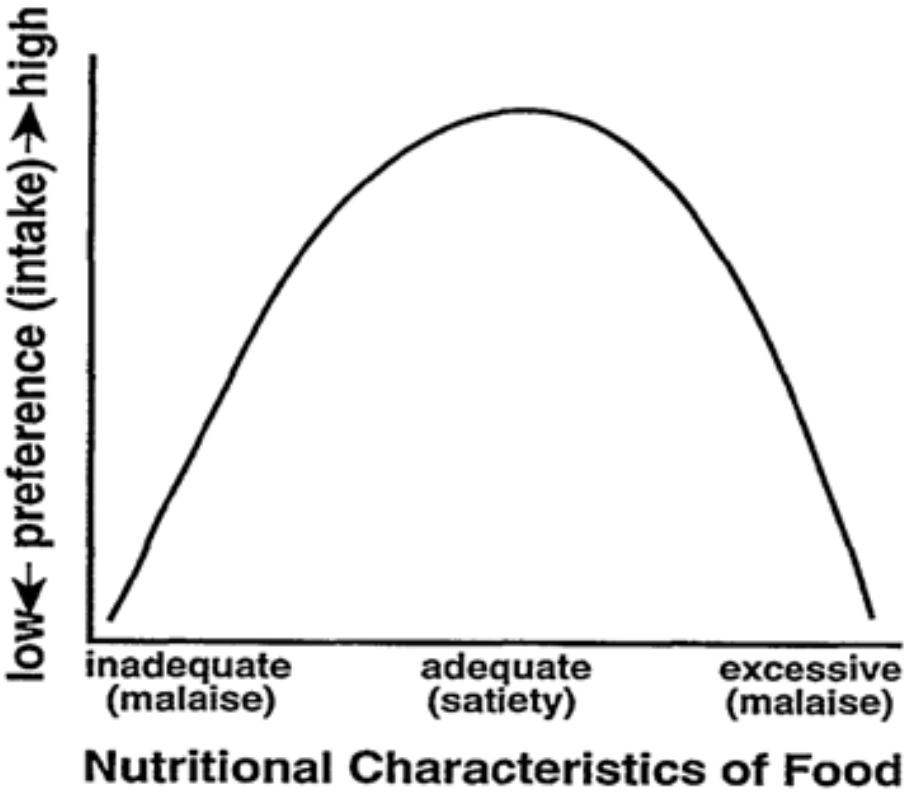
D'Eath *et al.* (2009) place particular relevance on the findings of Taylor (1977) in support of the possibility that hungry broiler breeders might prefer qualitative dietary restriction over quantitative dietary restriction. Taylor (1977) found that rats feed restricted to 80% of *ad libitum* fed bodyweight preferred low nutrient density (diluted with cellulose) pellets over high energy density pellets in an iso – energetic, limited quantity of pellets available, T – maze choice test. They also ran more slowly down a runway when suddenly switched from receiving 4 low energy density pellets (1.0 kcal/g) to 1 high energy density pellet (4.0kcal / g) despite the fact that the total caloric reward remained unchanged. However, rats offered unlimited access to both diet options in a two – pan choice test choose the high energy density diet (Taylor, unpublished results, cited in Taylor, 1977). This highlights the importance of the context and test methodology when assessing the dietary preferences of animals.

Although the findings of Taylor (1977) initially suggest a preference for quantity over quality it is important to note that factors other than energy density may have influenced this apparent preference. Energy density and quantity of pellets are confounded. The available literature suggests that animals value an apparent larger quantity over a small quantity even when the quality and actual quantity are identical. For example, chickens prefer 1 grain of rice cut into 4 pieces than 1 grain of rice (Wolfe and Caplan, 1941) and pigeons prefer lots of pellets that have a combined weight of 300mg over one 300mg pellet (Shettleworth, 1985; Capaldi *et al.*, 1989). This suggests that an animal's diet preference(s) may either reflect a proxy rule of thumb (Shettleworth, 1987) that more pellets means more food / energy or that other factors other than quantity may influence preference e.g. ease of handling pellets (Killeen *et al.*, 1993). Thus, one wonders if the Taylor rats would learn after a while that the options were iso – energetic and become indifferent to the options. This also raises the possibility that the broiler breeder may be initially 'fooled' into preferring one option on the basis of visual assessment of quantity. Thus the value of the preference test methodology when identifying the more satiating diet may be negated. However, this underlies the importance of quantifying any diet preference observed over a long time frame to enable changes in diet preferences to be quantified.

2.13. Satiety and diet preference

Provenza (1995) suggests that when it comes to diet preference and feed intake, preference and aversion can be seen sitting on a bell-curved continuum (see figure 1). Diets that are 'too satiating' are often preferred less than diets that are less satiating (Ackroff, 2008). To relate this to the experience of hunger: too little food results in hunger, consuming the 'right' amount results in the feeling of satiety, and continuing to consume additional food results in the feeling of 'excessive – satiety'. Excessive satiety (feeling 'over full') is aversive (Sclafani and Ackroff, 2004) and animals can develop conditioned aversions to flavours paired with consumption of high concentration (nutrient/energy dense) solutions (Sclafani *et al.,* 1994; Warwick and Weingarten, 1996; Warwick *et al.*, 1997). Further, infusions of cholecystokinin (a 'satiety' peptide) condition flavour preferences in low doses but flavour aversions in high doses (Perez and Sclafani, 1991). However, these studies investigate short term diet preferences and it is not clear if the same effect would be observed over the longer term in animals fed on a quantitatively versus qualitatively restricted diet.

Figure 1: Theoretical model of the bell-curved relationship between nutritional characteristics and diet preference (measured through intake) (Source: Provenza, 1995)



Related to this concept of excessive intake of nutrients is the concept of overconsumption of food following a period of food deprivation. Hertel and Eikelboom (2010) examined flavour aversions in rats feed restricted to 50% of *ad libitum* intake for eight days and then given 3 trials in which they had 24 hours exposure to saccharin solution. During this 24 hours exposure the rats were either maintained on, or released from, their feed restriction (either fully to allow the rat to compensate for over eating by binge eating or limited to a fixed quantity equivalent to that which a rat that had not been feed restricted would consume). A control group that were permanently on *ad libitum* feed access were also compared. The feed restricted rats that were allowed to consume significantly more food than either the control group or the rats given a fixed quantity (either 50% or 100% of *ad libitum* intake) and subsequently consumed significantly less saccharin in a two bottle preference test with water. This suggested that the rats experienced a flavour paired with over consumption of food as less pleasant. Unfortunately the rats that were allowed to binge during conditioning trials consumed less saccharin during conditioning trials also. It would be useful to see this replicated with measures in place to prevent this confound (i.e. offer a fixed quantity of saccharin below that which all groups voluntarily consumed during the conditioning trials). Bardos (2001), using a gut distension model to assess the effects of gut distension on conditioned flavour aversion, also found that rats consumed less of flavours associated with either volumetric or isometric distension of the large intestine. Further, studies indicate that increasing the volume of the gastrointestinal tract is associated with gastrointestinal discomfort (and pain if sufficiently distended), taste aversion and reduced intake (see a review by Bardos *et al.*, 2002).

At first glance this suggests that, if qualitative dietary restriction is more satiating that quantitative dietary restriction then the broiler breeders may show an aversion to flavours associated with qualitative dietary restriction dependent on where on the satiation curve (Figure 1) the bird sits. However, the converse may also be true as the feeding patterns associated with quantitative dietary restriction may induce binge-like feeding. Howie *et al.* (2009) found that 2 – 5 week old female broilers of a fast-growing strain reared on *ad libitum* rations had, on average 15.3 meals /24h period with 7.83g consumed at each meal. Nb. the data for line C (in Howie *et al.*, 2009) is reported here with an average weight of 1.9kg on day 35 as this most closely represents the target performance of female Ross 308 broiler breeders, Aviagen, 2007). By comparison, 6 week old broiler breeders can consume a daily ration (circa 42g) in 5 – 7 minutes under conditions of feed restriction (Savory and Maros, 1993) and utilise their crops to store this feed (Savory, 1985). Sandilands *et al.* (2005) in their

study comparing qualitative and quantitative feed restriction did not notice distended crops in the birds fed the qualitatively restricted diet (400g oat hull and 2.4 – 6% calcium propionate / kg of total feed) (Sandilands, personal communication). These combined observations suggest that feed restricted broiler breeders compensate by binging (eating more food at a meal than they would normally eat under *ad libitum* conditions).

Distension of any part of the gastrointestinal tract of the chicken is associated with a decrease in feed intake (Forbes, 2007). Thus, a bulky high fibre diet and / or excessive short term feed intake in regimes using quantitative dietary restriction may result in a volumetric change that causes satiety or discomfort. However, in contrast to mechanical distension of the gut (see: Bardos, 2001) the effect of voluntarily consuming low density, high volume diets on conditioned flavour preferences or aversions has not been studied.

2.14. Food deprivation and diet preference

Rogers and Blundell (1991) state that: "logic demands that need state (departure from some optimal value) must be detected and converted into a signal which *drives behaviour and adjusts preference* [my italics]". Thus, we should expect the dietary preferences of broiler breeders to be altered by chronic feed restriction. Food scarcity should increase the relative acceptance of a wider range of foods (i.e. to try and obtain sufficient energy and nutrition, (Orians, 1971, cited by Moons and Zeigler, 1979). However, the importance of the post-ingestive consequences of food [e.g. due to energy density] should also increase when the animal is in a negative energy state (Zyerev, 2004). Therefore, an animal is expected to be more discriminatory under conditions of hunger when two or more diet options are available.

Feed restriction affects the preference of animals for particular nutrients even when iso – energetic rations are offered (e.g. Warwick and Synowski, 1999; Kimura *et al.,* 2003; Perello *et al.,* 2010). Biomolecules (such as the hormone leptin) that signal the current energy state interact with metabolic pathways that affect relative hedonic value of a food (Stanley *et al*., 2005) probably drive these alterations in nutrient preferences. Comparatively short term feed deprivation can lead to increased taste sensitivity to sweet (humans, feed deprived for 14 – 16hours, Zyerev 2004; rats, feed deprived for 48 hours, Berridge, 1991) and salty substances (Zyerev, 2004). Thus, feed restriction might affect the relative sensory attractiveness of different nutrients dependent on the relative importance to the individual in that state (Zyerev, 2004) or by attenuating the relative importance of sensory aspects of a diet to the hungry animal.

Goldstone *et al*. (2009) compared the effects of images of high and low energy density diets on brain activity of humans that had either been deprived of food for 16h prior to an MRI scan or were tested satiated. Food deprivation biased areas of the brain responsive for the food reward systems in the direction of high calorie foods. However, total number of calories on show between the images was not controlled. Thus, it is not possible to identify whether the people were preferentially attracted to the increased energy density or were calorie tracking.

Satiated rats showed a preference for sucrose solutions over iso – energetic polycose solutions but this preference was modified in rats restricted to 85% of their bodyweight (Sclafani and Ackroff, 1993). Hungry rats showed a preference for complex carbohydrate (polycose and starch) over simple carbohydrates (sucrose), corn oil over sucrose but were indifferent between polycose and corn oil (but see Lucas and Sclafani, 1999, who found a preference for polycose over oil). The authors suggest feed restriction increased preference for several different food flavours [associated with respective macronutrient solutions] but also resulted in a relative decrease in the preference for sucrose (Ackroff and Sclafani, 1992). This was despite the fact that all the solutions tested were designed to deliver equal calories per millilitre ingested (0.8kcal/ml).

Rats offered a choice between iso – energetic solutions (2.3kcal/ml) that are either high-fat or high-carbohydrate show an enhanced preference for the high fat solution when they have been deprived of food for 24 hours prior to testing (Warwick and Synowski, 1999). Quantities consumed during training were restricted to 15ml per day of either solution to ensure that differences in consumption during training could not explain the findings. A preference for fat over sugar solutions was also found in rats restricted to 75% of bodyweight (Kimura *et al.,* 2003). Although feed restricted mice will form a conditioned place preference to an environment associated with either a high carbohydrate food reward or a high fat food reward (the food reward was iso – energetic between the types of food reward) they only continued to display the place preference for fat after 24h *ad libitum* feeding (Davis *et al.,* 2007).

Finally, Perello *et al.* (2010) used a dual rewarded conditioned place preference task that was associated with iso – energetic quantities of rat chow versus high fat pellets to investigate the effects of the 'hunger' hormone ghrelin on diet preferences of *ad libitum* fed mice. They found that mice injected with saline (the control group) failed to develop a preference. However, mice injected with ghrelin either before training (but not testing) or during testing in a conditioned place preference task developed a preference for the environment associated

with a high fat pellet (the alternative environment was associated with an iso – energetic quantity of rat chow). Crucially, the preference was strongest when mice were tested following an injection of ghrelin. Thus, by default, hunger is implicated both in selectively enhancing the ability of high energy density diets to condition place preferences and can also result in a deprivation – state dependent expression of preference under certain conditions. Most of these findings suggest that, in a two way choice test with iso – energetic high quality, low quantity versus low quality, high quantity diets broiler breeders are likely to show a preference for the high energy density diet option.

2.15. Chronic feed restriction and the avoidance of satiety?

The feeding behaviour of animals is adapted to managing energy balance in environments in which the availability of food quantity and quality may vary (Stubbs and Tolkamp, 2006). Thus, it is expected that state dependent preferences will reflect this adaptation. All macronutrients are not equal in their ability to influence satiety levels. The most satiating macronutrient is protein, the least is fat and carbohydrate is intermediary between the two (Stubbs and Whybrow, 2004). Chronic feed restriction seems to preferentially direct preferences towards the macronutrient fat (Warwick and Synowski, 1999; Kimura *et al.,* 2003; Perello *et al.,* 2010). This is interesting because fat is widely considered to be the least satiating of the macronutrients / more is consumed before the meal ends (Stubbs and Tolkamp, 2006) and rats gastric- infused polycose or fat show rapid short term depression of subsequent feed intake if infused polycose but delayed depression of feed intake if infused oil (Lucas and Sclafani, 1999).

One implication of this is that animals may prefer diets that are *less satiating* under conditions of food deprivation. The intake of food helps to restore the animal to a state of nutrient and energetic homeostasis. Thus, it makes sense to consume more food (either through larger meals or through more frequent meals or by a combination of both). Thus, hedonic shifts that increase preference for foods that delay the onset of satiation and reduce the duration of satiety in order to up – regulate daily caloric intake may be expected. However, it is expected that if this is the case, under conditions of chronic feed restriction, the broiler breeder will have to trade off any preference for 'less satiating' diets that allow it to consume more food before feeling 'full' against the negative feeling of feeling hungry as additional food is not available to make it more 'full'.

Support for the proposition that animals may prefer less satiating diets can be found in the observations that feed restricted rats prefer high fat foods or solutions to other diets or

solutions (Warwick and Synowski, 1999; Kimura *et al.,* 2003; Perello *et al.,* 2010). However, rats feed restricted to 87% of free feeding bodyweight, given a choice between iso – energetic solutions of polycose and oil, preferred polycose (Lucas and Sclafani, 1999). This indicates that a preference for high fat food is not always observed. The failure to ensure equal caloric intakes of each solution during one bottle conditioning trials may have influenced these results.

When short term satiety is artificially induced prematurely in rats by repeated injections of cholecystokinin (paired with meals), whilst reducing individual meal size, do not result in a reduction in bodyweight in rats who, instead, increase frequency of meals to defend their energy intake (see: Woods *et al.,* 1998). This suggests that, in animals, rapid satiation during meals is compensated for by up-regulation of meal frequency to maintain, or try to attain, energy homeostasis. The need for longer term diet preference studies is evident.

2.16. A case study: Prader – Willi syndrome and diet preference

Prader-Willi Syndrome (PWS) is a disorder caused by a chromosomal disorder that causes, among other clinical signs, mental retardation and hyperphagia in affected individuals (Donaldson *et al.,* 1994). When allowed *ad libitum* access to food, individuals may consume 5200 kcal (± 50) (see Donaldson *et al.,* 1994); thus, individual feed restriction to 1200 – 1600 kcal / day has been reported (Caldwell *et al.,* 1986). This represents 20 – 31% of *ad libitum* intake which raises some interesting parallels with the plight of the broiler breeder in which feed restriction may represent 33 – 45% of *ad libitum* intake (Hocking, 1993; Savory and Maros, 1993). PWS humans are not indiscriminate feeders (Caldwell and Taylor, 1983; Taylor and Caldwell, 1985; Fieldstone *et al.,* 1997). They show a preference for high carbohydrate foods (Fieldstone *et al.,* 1997). Further, when given a choice between a small quantity of preferred food and a larger quantity of a less preferred food, people with PWS are more likely to select the larger quantity of less preferred food than people without PWS (who are also, presumably, less hungry) (Glover *et al.,* 1996; but see Taylor and Caldwell, 1985).

Joseph *et al.* (2002) tested PWS and non-PWS people in an open economy task in which a choice between a small quantity now versus large quantity later of the same food was offered (with a time delay 15, 30, 60 seconds). They found that people with PWS showed a preference for the delayed delivery, larger option of food (irrespective of the length of the wait), whereas, people that were simply obese demonstrated no preference for one of the options. A follow up study by Joseph *et al.,* (2002) also found that, irrespective of whether a high value (defined as the preferred diet) or a lower value (less preferred food) was used in

the choice test, PWS people preferred the delayed larger option (treatment mean: > 80% for all foods tested). However, Caldwell *et al.* (1986) used food rewards as a reward for taking exercise in PWS people. They found that preferred rewards (high energy density) were more effective as reinforcers than un – preferred (low energy density) food rewards (both rewards were energy matched at 27 – 33kcal/ per reward). This suggests that the high energy density reward was perceived as more rewarding even though the volume of low energy density reward was presumably considerably more. However, this study utilised an open economy, short term, methodology. It is not clear whether these findings would apply in a longer term, closed economy, experiment in which the participants only received energy – matched portions of high and low energy density food (analogous to the broiler breeder situation). However, it would be interesting to replicate the research proposed in broiler breeders in human beings with PWS as, arguably, the chronic hunger experienced by people with PWS is as much a welfare issue as chronic feed restriction is in broiler breeders.

2.17. Drawing it all together: the importance of knowing why a broiler breeder might show a diet preference and what it may mean for welfare

Potential reasons why a broiler breeder may express a preference have been suggested and discussed throughout this review. Crucially, these include the relative palatability and satiating effects of each of the diets. However, there is another potential reason that should be mentioned. Kyriazakis and Tolkamp (2011) highlight that quantitative feed restriction also interferes with another of the five freedoms: freedom to express natural [feeding] behaviour. Whilst the bird is able to perform appetitive foraging behaviour throughout the 24 period, it is only able to consummate this behavioural sequence during the first few minutes post – being fed. Broilers [and other chickens] normally have small meals little and often (see: Howie *et al.,* 2009). Thus, broiler breeders have lost control over their normal feeding patterns (D'Eath *et al.,* 2009; Kyriazakis and Tolkamp, 2011). Loss of control is considered to be stressful for the animal (Wiepkema and Koolhaas, 1993). Qualitatively restricted diets (such as that described by Sandilands *et al.,* 2005) allow the bird to choose *when* and *how often* to feed. Thus, a broiler breeder may show a preference for the qualitatively restricted diet even in the absence of improved satiety. However, a 'bird in the hand is worth two in the bush'. Thus, chronically feed restricted and hungry broiler breeders may show a preference for a diet that allows a more rapid feeling of reduced hunger (albeit short lived) over one that normalises feeding patterns to those observed in *ad libitum* fed broilers (assuming the same diet option does not confer both benefits).

It should be clear from this review that many different, potentially competing, factors will affect any diet preference that the hungry broiler breeder may display during preference testing. Whilst "preference tests give a good first indication of how an animal feels about its environment" (Duncan, 2006, pg. 14) this does not tell the researcher *why* the broiler breeder prefers a particular diet option. Duncan (2002) claims that it is not necessary to know what the animal experiences just whether that feeling represents a positive or a negative affective state. Bateson (2004) argues that the most common criticisms of choice tests can be circumnavigated by arguing that, provided the animal remains healthy, then the welfare of the animal may be best served by still giving it what it wants, even if the choices it makes are not in its long term fitness.

However, to adopt this attitude is short sighted when trying to identify the preferences of broiler breeders for quantitative or qualitative dietary restriction. As noted at the start of the review, an animal's preference may be in response to immediate gain rather than long term preference (Dawkins, 1990) and be impulsive (Abyesinghe *et al.,* 2005). Or, alternatively, decisions that involve a more immediate gain may be preferred as they are adaptive in more naturalistic foraging situations (Stephens and Anderson, 2001). Although laboratory tests of this preference for immediacy normally focus on the delay between performing the appetitive (operant) response and receiving the reward (e.g. Abeyesinghe *et al.,* 2005) it is not clear how the animal will compare options in which both diets are immediately available (D'Eath *et al.,* 2005), albeit with varying 'handling' times per unit of energy.

The bird may show a Sophie's choice (Duncan, 2006) when constrained by immediacy that does not actually represent what the bird 'wants' (a key component of which, we assume with some justification by analogy, is to lessen or remove the subjective experience of hunger). Without an exploration of why the bird shows a preference or, if not, why not, it is possible that choices that a broiler breeder makes may actually worsen its welfare. Not just over some theoretical long term measure of 'fitness', but rather over the short term here and now of the commercially reared individual broiler breeders' daily existence. Thus, there is a need to quantify the reasons why a broiler breeder expresses any preference.

2.18. Conclusion and recommendations for further research

In conclusion, it is clear the diet preferences exhibited by animals are multifactorial. Animals (including humans) show preferences for diets based on the intrinsic properties of the food such as macro – and micro – nutrient composition and energy availability. However, the preferences exhibited are also affected by the nutritional and energetic state of the animal.

There is a dearth of studies that investigate how an animal, under conditions of feed restriction, trades off quantity versus quality in a closed economy, feed restricted, environment. Therefore, there is a need for research that focuses on this unique scenario for which there is unlikely to be an evolutionary precedent.

Preferences can be constrained by the context in which the animal is tested leading to choices based on short term benefits that may or may not be welfare friendly in the long run. This has implications for investigating hungry broiler breeder dietary preferences. Thus, it is proposed that an adequate exploration of whether a broiler breeder 'wants' to be a fed a diet that is qualitatively restricted or one that is quantitatively restricted must include a variety of approaches. These should be aimed at quantifying any want from various bird-centric perspectives. These include direct choice tests where the bird chooses between the two diet options as well as indirect approaches that focus on changes in the decision – making or cognitive biases that an animal shows when tested when hungry or satiated. Examples of this kind of decision making can be found in models of cognitive bias (see: Mendl *et al.,* 2009) and cognitive ecological models where the effects of energy balance affect preference shown either altering the optimal foraging strategy demonstrated (See: Kacelnik & Bateson, 1996b) or by leading to a violation of rational decision-making (e.g. Schuck-Pain, *et al.,* 2004). An adequate exploration of broiler breeder dietary preferences must contain elements of both in order to adequately understand the motivation and welfare implications of any preference observed.

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3. Experiment one: Quantifying Hungry Broiler Breeder Dietary Preferences using a Closed Economy T – maze Task

3.1. Preamble

This experimental chapter was published in the peer – reviewed journal Applied Animal Behaviour Science. The relevant reference is:

Buckley, L. A., Sandilands, V., Tolkamp, B. J. and D'Eath, R. B. (2011) Quantifying hungry broiler breeder dietary preferences using a closed economy T – maze task. *Applied Animal Behaviour Science* 133(3): 216 – 217

Aspects of this study have also been presented at one conference. The relevant reference is:

Buckley, L. A., Sandilands, V., Tolkamp, B. J. and D'Eath, R. B. (2011) Quantifying hungry broiler breeder dietary preferences using a closed economy T – maze task. *44th Conference of the International Society for Applied Ethology,* 4th – 7th August, Uppsala, Sweden. [oral presentation]

3.2. Abstract

This study aimed to identify hungry broiler breeders (n = 12) preferences for quantitative (Control) or qualitative dietary restriction (QDR) in a closed economy environment. The QDR option was either 3 g calcium propionate/kg total feed (n = 6) or 300 g oat hulls/kg total feed (n = 6). Quantitatively restricted or QDR portions ensured equal growth regardless of choice. Birds were separately taught a Control diet versus no food and a QDR diet versus no food task to allow each diet's satiating properties to be learnt. Birds had to associate the T-maze coloured arms with dietary outcomes to immediately obtain food. Birds learnt this task easily ($p<0.001$). A choice between the Control diet and the QDR diet was then offered but neither group demonstrated a diet preference. Study modifications demonstrated this was not a failure to discriminate between the diets per se (the Control diet was strongly preferred under *ad libitum* conditions ($p<0.001$)) or novel colour combination confusion (the colour associated with food was immediately selected when two novel food versus no food colour combinations were offered ($p<0.001$)). Most birds still failed to show a significant preference when the Control diet quantity was increased by 50% to make it 'obviously' bigger and better. Therefore, it was concluded that the failure to show a dietary preference was due to task learning failure and not necessarily lack of dietary preference. Where a preference was observed it was always for the control diet. Possible reasons for this failure to learn are discussed.

3.3. Introduction

Freedom from hunger is one of the five freedoms necessary for good welfare (FAWC, 1998). Hunger is 'a negative affective state' (D'Eath *et al*., 2009), associated with suffering for the animal involved (Dawkins, 1990). However, for broiler breeders (the parent stock of meat chickens) selectively bred for fast growth (and therefore large appetites), preventing hunger by *ad libitum* feeding causes obesity and severely compromises physical health and fertility (Hocking *et al.*, 1987; Robinson and Wilson, 1996). Consequently, optimising growth through quantitative feed restriction is integral to management in the industry. Birds are fed 25 – 50% of *ad libitum* intake *(*Savory *et al*., 1993). Behavioural and/or physiological stress indicators are apparent (Hocking *et al*., 1993; Hocking, *et al.,* 1996; de Jong, *et al.,* 2002; de Jong, *et al.*, 2003) with general acceptance that these birds experience chronic hunger (Mench, 2002; de Jong, *et al.,* 2003).

To address this welfare issue, researchers have attempted to reduce hunger by adjusting the commercial ration quality either by adding non – low – nutritive fillers to make the diet more bulky and / or by adding appetite-suppressing compounds (Hocking and Bernard, 1993; Savory, *et al.,* 1996; Nielsen, *et al.,* 2003; Hocking, *et al.,* 2004; Sandilands, *et al.,* 2005). This is called qualitative dietary restriction (QDR). It is possible with this method for birds to be fed *ad libitum*, meet commercial growth rates and be healthy and fertile by adding increased levels of calcium propionate (CAP) (appetite-suppressing compound) and fixed levels of oat hulls (fibrous filler) to the commercial ration (Tolkamp *et al*., 2005).

Unfortunately, QDR effects on behavioural and physiological indicators of hunger stress in feed restricted broilers are mixed (Savory *et al.*, 1996; Savory and Lariviere, 2000; Nielsen, *et al.,* 2003; de Jong, *et al.,* 2005; Sandilands, *et al.,* 2005, 2006; Hocking, 2006) and studies are inconclusive. A voluntary reduction in overall energy consumed (compared with *ad libitum* intake of a regular commercial feed) or consumption rate is not necessarily indicative of, or synonymous with, reduced hunger. Birds may consume less energy because they are satiated (a positive welfare outcome) or they may eat less or more slowly because they find the diet aversive (a negative welfare outcome). Further, while combining CAP and oat hulls has synergistic effects on reducing energy intake (Tolkamp *et al.*, 2005), one compound may be aversive whilst the other satiety-enhancing. Thus, interpreting differential rates of consumption and other behavioural indicators is difficult (D'Eath *et al*., 2009). Consequently, additional methods of quantifying the potential benefits of feeding QDRs are needed.

Choice tests are a novel way to navigate round this interpretive difficulty (D'Eath e*t al.*, 2009). Choice tests are widely used in evaluating animal welfare and assume an animal's preferred option would lead to enhanced welfare. Dawkins (2004) claims only two questions need answering when evaluating an animal's welfare: Is it healthy? Does it have what it wants? Healthy broiler breeders can be produced on a typical quantitative restriction diet or on a QDR (Tolkamp *et al*., 2005). Therefore, the remaining question is: do feed restricted broiler breeders prefer this feed restriction to be quantitative or qualitative?

This study's primary aim was to investigate feed-restricted broiler breeder (*Gallus gallus domesticus*) preferences for either quantitative feed restriction or a QDR using a closed economy T-maze colour-diet association and discrimination task. Two different compounds – CAP and oat hulls (FIBRE) – were tested separately in choice tests (commercial diet versus experimental diet) in case of conflicting effects on affective state and thus preference. When initial results suggested no emerging significant preference, the experiment was modified and further conditions were imposed to determine whether the results reflected genuine indifference or a failure to learn the task. The specific hypotheses tested are outlined separately in the relevant experimental modification sections. Thus, it should be noted that the experimental design, results and initial discussion are described in two sections: firstly, the original study design and, secondly, the subsequent experimental modifications.

3.4. Methodology

3.4.1. Subjects

This study used 24 female Ross 308 broiler breeders, obtained as day old chicks. Birds were randomly allocated to one of two treatment groups at 35 days. These groups were 1) Control diet versus CAP diet (CVC, n = 12), and 2) Control diet versus FIBRE diet (CVF, n = 12). Between groups the experimental protocol was identical except for the diets fed from day 35 (see section 3.4.5.2., start of two-pan choice test). Before beginning the T-maze choice experiment (day 42), group-size was reduced (n = 6 per treatment group) by euthanizing the three heaviest and three lightest birds within each treatment group. Group size was reduced for the second part of the study for practical reasons (equipment and labour availability).

3.4.2. Housing & husbandry

Birds were reared according to the producer's recommendations for lighting and heating (stepwise lighting and heating reductions ~ 23 to 8 hours light from day 11 and 31ºC to 20-22ºC from day 25 respectively) (Aviagen, 2006). Birds were group – housed according to body weight in three groups (n=8) until day 14, then eight smaller groups (n=3) until day 35. To aid growth management, birds were occasionally switched between groups to ensure similar bodyweight birds were housed together. On days 28 - 34, the birds were fed separately and then returned to their group. Birds were housed and tested in the same room. However, birds were housed in different pens from their test pens to ensure pen familiarity did not influence choice test behaviour. From day 35, birds were housed individually in test pens (9am – 5pm) and group-housed in home pens overnight. All pens were 1m x 1m, contained wood shavings and provided ad libitum water access. Home (group) pens were cleaned weekly. Test pens were cleaned as needed.

3.4.3. Nutrition & feeding

3.4.3.1. Growth curves

Bird growth rate (until week 12) was modelled on the producer recommended Ross 308 broiler breeder growth curve for 5% egg production at 25 weeks (Aviagen, 2007) but slightly exceeded this recommendation post the change to the mash diet. Target weight gain (weeks 5 – 12) was 100 g / week. Actual weight gain was an average ((± standard deviation) 119 g /

week (± 12.1 g). Producer guidelines state feed levels once increased should never be decreased (Aviagen, 2006). Further, sudden diet quantity changes could have affected the birds' learning about diet-satiating properties. Therefore, although bird growth rate was slightly too fast, this trajectory was maintained.

3.4.3.2. Starter diet and protocol

From day 1 – 34, birds were fed a commercial diet (Laser SP starter Crumb, BOCM Pauls Ltd., Ipswich, Suffolk). Birds were individually fed additional feed if necessary to ensure actual bodyweight was close to producer target weight and coefficient of variation between birds was minimised.

3.4.3.3. Experimental diet and protocol

From day 35, birds were fed two diets (see below for feeding/exposure to diets protocol). The control diet (both treatments) was a custom-made grower mash (Target Feeds Ltd., Whitchurch, Shropshire) and this was also the basis for both experimental diets. The mash diet supplied 150 g crude protein and 11.5 MJ ME per kg of food. The CAP diet was the mash diet plus 30 g Calcium propionate / kg total feed. The FIBRE diet was the mash diet plus 300 g finely-ground (4mm) oat hulls / kg total feed. Each experimental diet portion was equivalent to the control diet portion (g) plus the respective addition. The calcium propionate was supplied as Luprosil ® salt (BASF, Germany).

Diet rations were designed to ensure equivalent growth, based on Tolkamp *et al*., (2005) who found that the quantity of basal feed (commercial feed minus CAP and OH) consumed *ad libitum* by their QDR birds was similar to birds fed the commercial feed restricted ration. Initial dietary preferences were also investigated as initial dietary preferences are modifiable by post-ingestion feedback (Provenza, 1995; Forbes, 1998; Kyriazakis, *et al.*, 1999). Quantities of the compounds added were less than in Tolkamp, *et al.* (2005). This reflected previous unpublished findings by the authors that indicated that gradual adjustment to QDR may mean insufficient energy consumption initially if compound inclusion levels are high. Broiler breeders are sensitive to restriction severity (Savory *et al.*, 1993; Bokkers and Koene, 2004; Bokkers *et al.*, 2004). Thus, we assumed, they should prefer an increase in satiety, even if that satiety is not complete.

3.4.4. Experimental apparatus

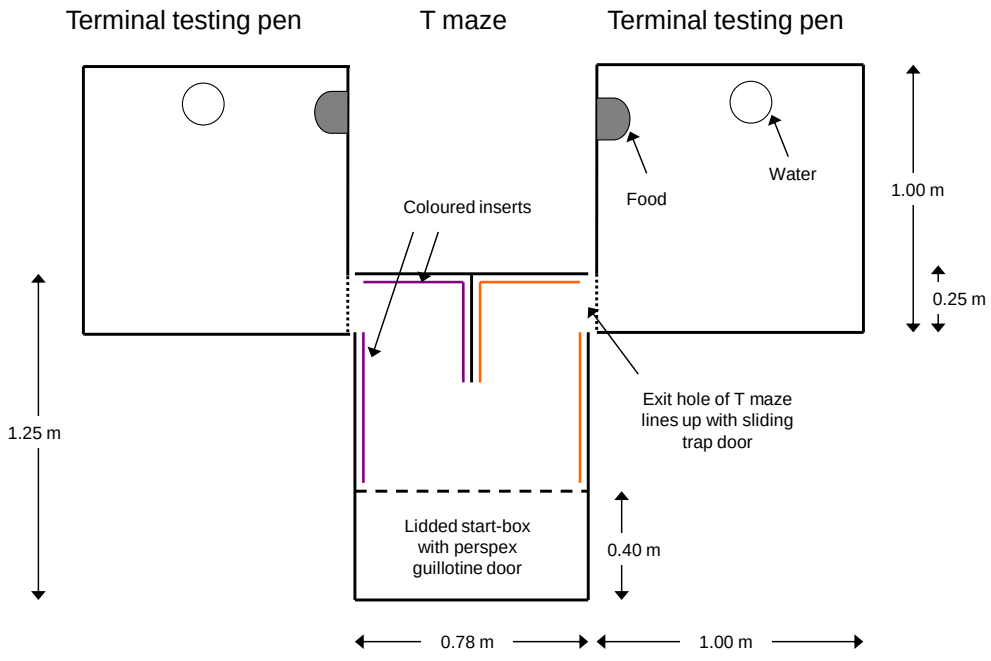
3.4.4.1. Two – pan choice test – initial dietary preference experiment

Test pens (1m x 1m) were solid-sided to prevent visual access to other birds. Food was provided in D – cup feeders (11.25cm (l) x 6.25cm (w) x 8.75cm (d)). These were attached to the pen front 10 – 12 cm apart. The water bowl was on the floor in the middle of pen.

3.4.4.2. T – maze choice test experiment

The experimental apparatus comprised two sections: the T – maze and the two terminal testing pens that the T – maze arms exited into (Figure 1). The T – maze was of wooden construction. Interchangeable coloured wooden inserts slotted into each T – maze arm (right/left/end). The maze height was 40cm. Terminal test pens had a guillotine hatchway situated on the front left of the pen (25cm x 25cm). The D – cup feeder location ensured its contents were only visible once the bird had entered the terminal pen. The terminal pens were the same as the pens used to house the birds outside of the test situation and during the initial dietary preference experiment. However, to prevent familiarity biasing preference, the individual birds were not tested in the pen(s) they had previously experienced.

Figure 1: Experimental set-up utilised in the T – maze choice test experiment. Additional terminal testing pens are omitted for clarity.



3.4.5. Training and testing

3.4.5.1. Handling and socialisation

To reduce the potential effects of stress, birds were socialised and habituated to potential environmental stressors by being handled several times a day (10 – 120s) and by gradually increasing isolation from other chicks. The latter was initially synonymous with handling (as above) then involved separation of individual chicks by solid barriers and allowed to find their way around the barrier to return to their group (Day 8 onwards, 10 – 60 sec, 1 – 5 times / day, 3 times / week) and, finally, by daily solitary feeding (day 28 – 35). Solid barrier use encouraged exploratory behaviour to reduce the risk of fear or anxiety that might affect performance during the later T-maze training/testing.

From day 21, birds were group-introduced to the T – maze and released into the arms to explore (for 15 minutes / twice daily; three times / week). From day 28, birds individually explored the T – maze and adjacent pens (for 15 minutes / once daily three times / week). Finally, a radio played daily habituated birds to human voices/noise and to mask unwanted facility sounds.

3.4.5.2. Two – pan choice test – initial dietary preference experiment

During days 35 – 41, the primary aim was diet habituation before training/testing as dietary neophobia reduces intake in fowl (Murphy, 1977). However, it also allowed investigation of initial dietary preferences prior to potential preference modification by post-ingestive effects.

Birds were given equal exposure to both the control and experimental diets. Both diets were offered simultaneously (each portion equalled 1/4 of total daily feed provided) with two feeding opportunities / day (09:00 h and 13:00 h) for 7 days. The rations offered at each feeding opportunity over this period were: control diet: 11g; CAP diet 11.3g and FIBRE diet: 15.7g. Individual feed intake was measured twice daily for the first 5 days. The food removed, weighed, and returned after 10, 20, 30, 40, 50, 60, 120, 180 and 210 min. Food left after 240 min was removed, weighed and discarded. Diet was balanced (within and between birds) for pen side and randomly switched sides between feeding opportunities.

3.4.5.3. T – maze choice test experiment

3.4.5.3.1. <u>General testing protocol</u>

Each bird was given five T – maze trials / day (90 min apart). Within treatment group, birds were tested in the same order each trial. Within trial, all birds in a treatment group were

tested before the other treatment group birds were tested. This was done for practical reasons as alternating between birds from different treatment groups would have increased the time taken to test all 12 birds. The group tested first alternated daily. Each bird obtained 1/5 of her daily feed ration at each trial. No further food was available.

3.4.5.3.2. Dietary contingencies associated with colours

The aim of this training was for birds to associate coloured T – maze arms with different dietary outcomes. Different colours were used for the treatment groups as the experiment was originally planned as a crossover design. Necessary experimental modifications prevented this crossover and it is not referred to further. The colours used were balanced within food versus no food stages for dietary contingencies. Technical and sample size reasons prevented all colour combinations being balanced. Therefore, only stages at which initial colour biases may have affected learning were balanced. It was assumed any initial biases would have been modified by experience by the experimental versus control diet stage. The colours used were as follows:

CVC group: 1) Control diet versus no food task: green versus yellow (balanced for diet option: colour; hereafter B); 2) CAP diet versus no food task (B): purple versus orange; 3) CAP diet versus Control diet: orange (CAP diet) versus green (Control diet) OR purple (CAP diet) versus yellow (Control diet).

CVF group: 1) Control diet versus no food task: red versus black (B); 2) FIBRE diet versus no food task: white versus blue (B); 3) FIBRE diet versus Control diet: red (FIBRE diet) versus blue (Control diet) OR black (FIBRE diet) versus white (Control diet).

3.4.5.3.3. T – maze training protocol

The general procedure for each trial was as follows: at the start of the day, the T – maze was placed in the runway between the two parallel rows of 'terminal' testing pens. The distal exit holes at the end of the T – maze arms were lined up with the guillotine hatchways (which were secured open) of the end two pens. The appropriate coloured inserts were attached to the appropriate arms of the T – maze. The bird allocated to these pens was collected from its home pen, placed in the start box and held for 30 seconds. The Perspex door was then lifted and the bird was allowed to walk through the runway apparatus and exit into either terminal pen. The bird was then closed into this pen. How long the bird was held in this pen depended on the task and is described below in the food versus no food task and experimental diet versus control diet section. Once the trial was completed, the bird remained in the terminal

pen until it was re – tested (circa 90 minutes). To allow further birds to be tested during this period, the T – maze was then moved along the walkway to line up with the next set of terminal pens and the next bird tested. This procedure was repeated until all six consecutive pairs of terminal pens had been used. The same procedure then took place in the second walkway and the second set of parallel pairs of 'terminal' pens. All the birds from one group were trialled in the same walkway / set of pens (i.e. CVC birds occupied the pens in walkway one and CVF birds the pens in walkway two).

3.4.5.3.4. Food versus no food task

Birds were initially given 35 trials (seven consecutive days) per diet (phases 1 and 2) to learn separately about the post-ingestion ingestion feedback effects of each diet, and to learn to associate a certain colour with each diet. In phase 1, half the birds were randomly allocated to be trained with the control diet vs. no food, while the remainder were trained with their experimental diet (FIBRE or CAP) vs. no food. In phase 2, each bird then learnt the other contingency. Birds were trained in a discrimination task between colour X = food and colour Y = no food. If the bird made the wrong choice (i.e. it selected the pen containing no food) it was held in its chosen pen for 1 minute before the hatchway was raised and the bird allowed to re – enter the T – maze. The hatchway was then closed behind the bird, in effect forcing it to choose the correct (food rewarded) pen. Once it had entered this pen the trapdoor was closed behind it and the bird was allowed to consume the food. The bird then remained in this pen for approximately 90 minutes (until the next trial).

Immediately after phases 1 and 2 had been completed, the birds were given twenty 'refresher' trials per diet (experimental diet versus no food and control diet versus no food tasks) to remind them of the post – ingestion effects of each diet and the colour – diet type association (phases 3 and 4). The diet the birds experienced in phase 1 was offered to them in phase 3 and the diet offered to them in phase 2 was offered to them in phase 4. This re – representing of the diet – colour combinations ensured that the birds had retained the information learnt after a period of time not exposed to the diet – colour combination as we were concerned the association might have extinguished without regular reinforcement and this would affect any preference seen.

The first fifteen of these trials for each diet were consecutive (i.e. phase 3 was five trials per day for 3 days of one diet then the same procedure was followed for phase 4). The last five trials of each phase were organised (five per day over 2 days) such that the task was

alternated between the experimental diet versus no food task and the control diet versus no food task (five trials per diet spread over 2 days). These final 2 day period data were analysed as though it was a fourth day of phases 3 and 4.

3.4.5.3.5. Experimental diet versus control diet (phase 5)

After the food versus no food training had been completed, birds were given ten trials (over 2 days) in which they could choose between a portion of control diet and a portion of experimental diet. The procedure was as described above for the food / no food task but with one exception: there was no 'wrong choice' and birds remained in the pen they selected first. This phase had been planned to last 35 trials (7 days) but ended early after ten trials due to the unexpected behaviour of the birds (see results, section 3.5.2.3.).

3.4.6. Statistical analysis

Unless otherwise stated in the results section(s), all statistical tests were performed using Genstat (Version 11.1, VSN International Ltd., Hemel Hempstead, UK).

3.4.6.1. Two-pan choice test – initial dietary preference test

Only the first 10 min of feed intake was analysed due to rapid consumption. After this point, for all birds, total (both diets) intake approached 100% rendering preference quantification meaningless.

Data were initially expressed as intake of each diet as a proportion of total intake during each session**.** However, the transformed data (arc-sine transformation), were neither normal (Shapiro-Wilk normality test) nor homogeneous (Barlett's Test for variance homogeneity). Thus the proportional intakes were analysed non-parametrically using the Kruskall-Wallis (within treatment between day comparisons) and Wilcoxon Matched Pairs test (comparisons between average daily consumption of each diet by each bird).

3.4.6.2. T – maze choice test experiment

For all phases of the T – maze choice experiment (including subsequent modifications to the study design), a Generalised Linear Mixed Model (GLMM) was used to investigate the following fixed effects: treatments, phases, days, colour-combinations (random effect: bird/trial) and bird (random effect: side) and to generate logit-transformed predicted means (group daily and overall mean). The response variate used for all analyses was 'diet option chosen'. Where the GLMM could not model the data using the F-ratio (F) the Wald statistic (W) is reported. Post-hoc group analyses of differences from 0.5 (i.e. no preference shown)

were manually calculated using χ^2 to compare for differences from 0 at 1 degree of freedom using a Chi-squared (χ^2) – distribution table (Petrie & Watson, 1999). The test statistic (T) used for this was:

T = (predicted mean / S.E. of the predicted mean)^2

Individual bird differences from 0.5 were calculated using binomial probability distribution tables.

Side bias severity scores were calculated by blocking data into groups of 10 consecutive trials. The blocks of data used were: phases 1 and 2 (first 10 trials), phases 3 and 4 (last 10 trials) and phase 5 (all ten trials). From these data an individual bird score was calculated. 10/10 and 0/10 represented 100% preference for the right and left side respectively. To convert to a severity score (independent of preferred side), each bird's ten-trial score was reassigned a new 'side bias severity' score (0 – 5, 5 being the severest bias possible):

Original score	0	1	2	3	4	5	6	7	8	9	10
New score	5	4	3	2	1	0	1	2	3	4	5

This data were analysed using the Kruskall-Wallis test. Pair-wise post-hoc testing was performed using the Mann-Whitney U Test. The pairs tested were phase 5 versus phase 1 (first 10 trials), phase 2 (first 10 trials), phase 3 (last 10 trials) and phase 4 (last 10 trials).

3.4.6.3. Modifications

Unless otherwise stated within the results section, data were analysed as in section 3.4.6.2.

3.4.7. Ethical considerations

This study was carried out under the Animals (Scientific Procedures) Act 1986 and approved by the Scottish Agricultural College's and Roslin Institute's ethics committees. The Home Office Code of Recommendations for the housing of poultry was met or exceeded at all times. Birds were euthanised by an approved Schedule One method (barbiturate anaesthetic overdose).

3.5. Results (1)

3.5.1. Two – pan choice test – initial dietary preference experiment

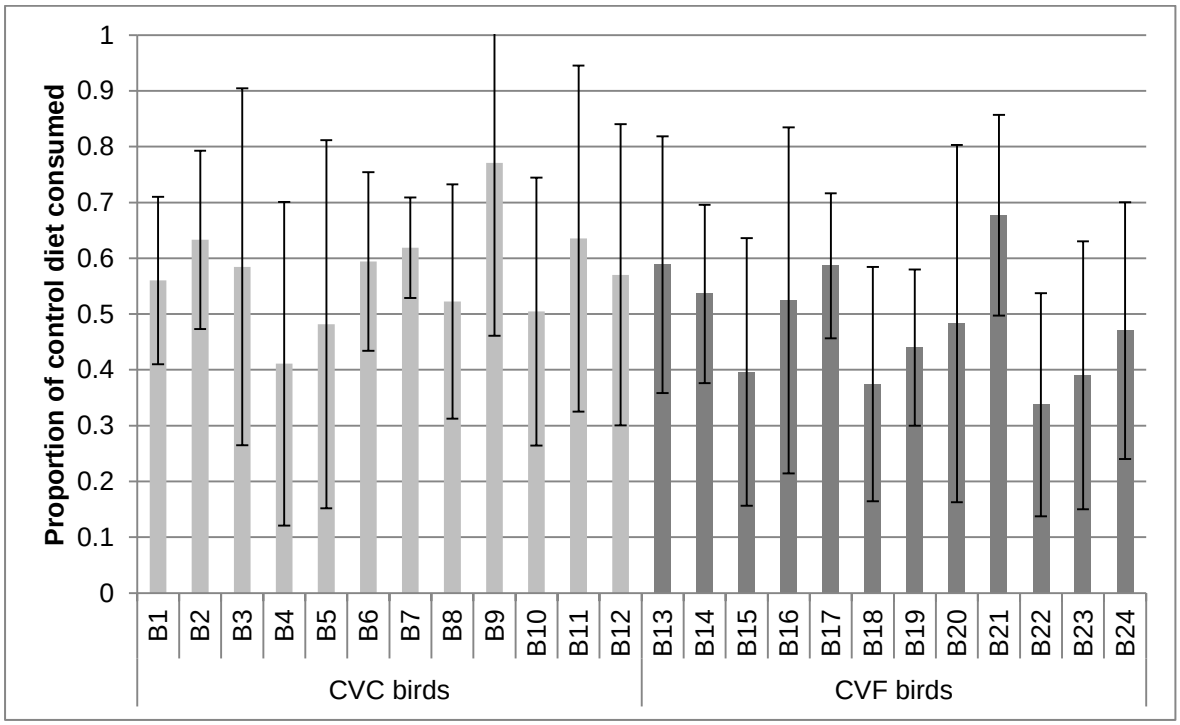
3.5.1.1. Control diet versus CAP diet (CVC)

Overall, the birds showed a preference for the control diet (W = 9, n = 12, P = 0.016), based on the individual mean intake of 12 birds over 10 occasions (5 days observations). The mean % intake of the control diet by the birds was 57%. However, there was considerable variation within-bird between the different tests (mean standard deviation of within-bird variation in control diet consumed as a proportion of total intake in a session = 0.25) (see: figure 2). There was no significant effect of bird, day or session (AM/PM).

3.5.1.2. Control diet versus Fibre diet (CVF)

Overall, the birds did not express a preference for either diet (W = 31.0, n = 12, P = 0.569), based on the mean intake of 12 birds over 10 occasions (5 days observations). The mean % intake of the control diet by the birds was 49%. However, there was considerable variation within-bird between the different tests (mean standard deviation of within-bird variation in control diet consumed as a proportion of total intake in a session = 0.23) (see: figure 2). There was no significant effect of bird, day or session (AM/PM).

Figure 2: Mean individual bird intake of the control diet during the initial two-pan choice test expressed as a proportion of total diet (experimental + control) consumed within 10 minutes. The errors bars indicate the standard deviation for the within-bird variation across data points. Data was collected on 10 separate occasions (2 sessions per day for 5 consecutive days).



3.5.2. Food versus no food discrimination trials

3.5.2.1. Initial 'learning' trials (phases 1 and 2)

Overall, analysed at the group-level, birds in both groups showed a preference for the colour associated with food in the food versus no food trials (CVC treatment group: phase 1: $\chi^2 = 21.19$, d.f. = 1, $p < 0.001$; phase 2: $\chi^2 = 43.54$, d.f.1, $p < 0.001$; CVF treatment group: phase 1: $\chi^2 = 17.89$, d.f. = 1, $p < 0.001$; phase 2: $\chi^2 = 48.22$, d.f. = 1, $p < 0.001$). There was a significant effect of day ($F_{(6,823.0)} = 15.89$, $P < 0.001$) with birds picking the food option significantly more often than the no food option during the last few days of phases 1 and 2 indicating that they had learnt to associate the colour with food (Figures 3). There was also an effect of phase ($W_{(1,7.27)} = 7.27$ $P = 0.007$) with birds showing a stronger preference for the food over the no food option in phase 2, which indicated that they found the task easier to learn the second time. There were no other significant effects or interactions (including diet option offered). In phase 1, 10/12 birds (5/6 in each treatment group) individually performed better than chance in the last 20 trials ($\geq$ 15/20 choices for the food option $p \leq 0.041$). The remaining birds selected the correct option 14/20 times. In phase 2, all birds met this criterion over the last four days.

3.5.2.2. 'Refresher' trials (phases 3 and 4)

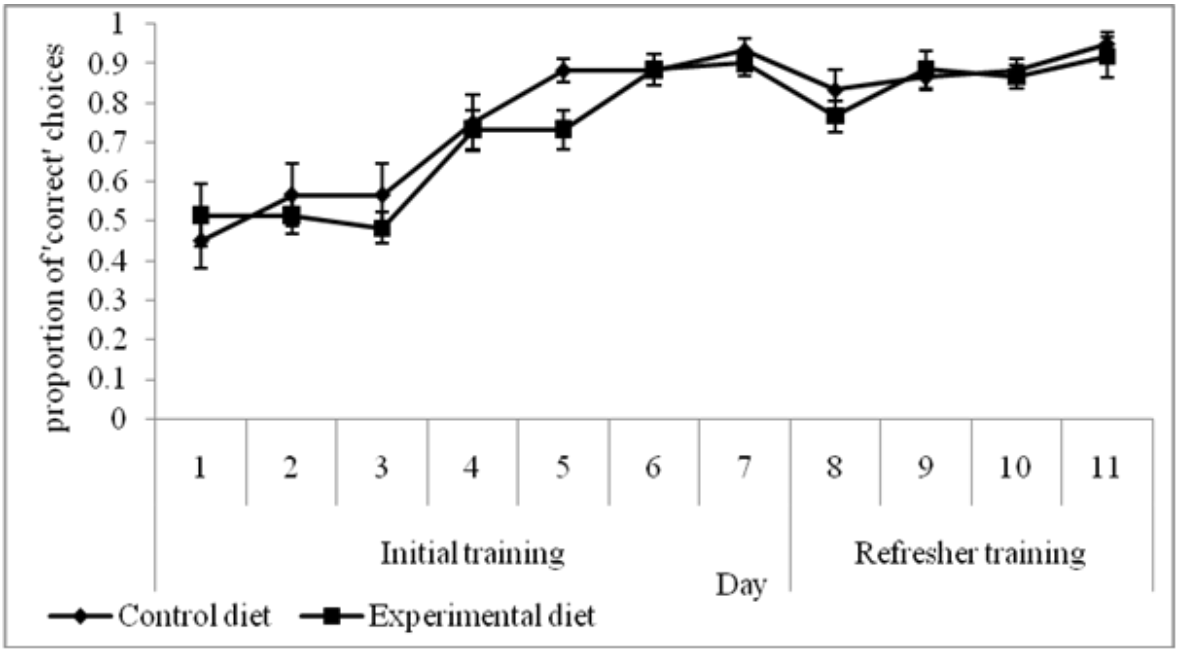
Overall, both treatment groups showed a preference for the colour associated with food in the food versus no food refresher trials (CVC treatment group: phase 3: $\chi^2 = 36.19$, d.f. = 1, $P < 0.001$; phase 4: CVC: $\chi^2 = 43.13$, d.f. = 1, $P < 0.001$; CVF treatment group: phase 3: χ^2 36.2, d.f. = 1, $P < 0.001$; phase 4: $\chi^2 = 49.67$, d.f. = 1, $P < 0.001$). This indicated that they had retained both the colour – food / no food associations after a period of 4 – 7 days of no exposure to each combination (whilst the other combination association was being trained / refreshed).

There was a phase effect ($F_{(1,460.0)} = 6.08$, $P = 0.014$), with birds in both treatment groups performing better in phase 4 than in phase 3. However, all birds individually performed better than chance in each of the 'refresher' phases (≥15/20 choices for the food option, $p \leq 0.041$).

An effect of day was also apparent ($F_{(3,460.0)} = 3.02$, $P = 0.030$) with birds increasingly picking the colour – food option over time in phase 3. However, irrespective of phase, both treatment groups showed a significant preference for this option shown from day 1 (Figure 3). There were no other significant effects or interactions (including diet option offered).

Figure 3: Food versus no food trials: proportion of 'correct' choices by diet (experimental or control option) × trial day. Data for both treatment groups has been combined, as there was no significant difference between the treatment groups in terms of learning the food versus no food task (irrespective of diet option). Hence, experimental diet refers to both the CAP diet and the FIBRE diet. A preference for the food option (X = 4.31, d.f. 1, $p < 0.05$) was observed on days 5, 6, 7, 8, 9, 10, 11 (control diet) and days 6, 7, 9, 10, 11 (experimental diet). Error bars = s.e.m. Figure legend: closed diamond = control diet; closed square = experimental diet.

† Although a continuous line is drawn through days 1 – 11 to aid clarity, the reader is reminded that birds had a 4 or 7 day break between day 7 and day 8 to allow the other diet – colour combination training (initial and / or refresher) to occur. Day 11 (phase three and four) is a composite day and actually took place over two days as the last five trials for each were alternated by trial.



3.5.2.3. Experimental diet versus control diet (phase 5)

At the group level no diet preference was observed (CVC: $X^2 = 0.04$, d.f. = 1, $P > 0.1$; CVF: $X^2 = 1.8$, d.f. = 1, $P > 0.1$) and only one bird showed a significant diet preference (9 out of 10 choices were for the control diet option, $p < 0.05$). However, birds in both treatment groups showed side biases with 3 out of 6 birds in each treatment group showing a significant side bias (9 out of 10 choices for a specific side, $p < 0.05$) and a further 4 birds selecting a specific side 8 out of 10 times. There were no other significant effects or interactions on either diet or side preferences. Therefore, data from both groups was combined in the analysis of side biases observed.

A comparison between any potential side biases observed in Phase 1 (1st ten trials), phase 2 (1st ten trials), phase 3 (last ten trials), phase 4 (last ten trials) and phase 5 (control versus experimental diet; all ten trials) indicated that there was a significant phase effect (H = 26.59,

d.f. = 4, P < 0.001). Individual birds picked the same side pen on repeated trials significantly more often in phase 5 compared to any other preceding phase: phase 1 (1^{st} ten trials), U = 17.0, n = 12, P < 0.001; phase 2 (1^{st} ten trials), U = 26.0, n = 12, P = 0.003; phase 3 (last ten trials), U = 10.0, n = 12, P < 0.001; phase 4 (last ten trials), U = 4.0, n = 12, P < 0.001

3.6. Discussion (1)

3.6.1. Initial dietary preferences

The results indicated that CVC birds showed a small preference for the control diet and CVF birds did not show a preference. This suggested the CAP diet was initially less liked than the FIBRE diet or the control diet. One possibility for the failure to show any or strong preferences is that the birds consumed almost all the entire total ration (control diet plus experimental diet) within 10 minutes and thus any preference was hidden. However, an analysis of the data (not reported here) in which any data point in which the bird had consumed more than 75% of the total ration (CVC birds) or 60% of the ration (CVF birds) was excluded from the analysis obtained the same direction of preference (CVC group) or lack of preference (CVF birds) reported here with minimal, non-significant effects on strength or direction of any preference. Different 'cut off' points were selected for each treatment group in this alternative analysis due to the quantity of experimental diet being different between the two groups. However, the small quantities of food offered remained a serious limitation that potentially affected interpretation of the findings as the strength of any potential preference was artificially truncated. When data was excluded where consumption exceeded the cut off threshold, whilst data was still available for analysis on at least two sessions per bird the median number of sessions per bird that were included in this alternative analysis was considerably reduced (CVC: 7; CVF: 5). This illustrated how close the birds were to fully consuming the feed ration during the first ten minutes. It was not possible to offer true *ad libitum* conditions due to this being a preparatory phase for the main experiment. With hindsight, one daily feeding session would have benefited data collection in view of the rapid feed consumption.

Within bird, the proportion of control diet consumed varied considerably between feeding opportunities. Anecdotal observations suggested that this was because birds stuck with the first bowl of food they encountered and stayed with this bowl until most of that ration was consumed. This may reflect diet type indifference. However, it may also reflect hunger state. High motivational drive to rapidly consume any food found might initially have suppressed motivation to obtain a more favourable food source. At five weeks feed restriction is already severe: on day 35 birds reared conventionally weigh an average of 560g and are fed circa 44g / day. This is considerably less than an *ad libitum* fed broiler breeder would consume on day

35 (average 159.8g/day consumed) or with an average bodyweight of 577g (average 93g/day consumed) (unpublished research by authors). This may have seriously impeded the exhibition of preferences.

In summary, the approach taken was not useful for evaluating sensory – led initial dietary preferences due to insufficient food quantities offered and the nature of the birds' feeding behaviour.

3.6.2. Performance during choice test

Birds found it easy to learn a food / no food discrimination task and they were able to retain this information. However, they then failed to show a diet preference in the control diet versus experimental diet choice test. Side preferences more clearly explained bird performance than diet preference. It was unclear whether the development of the side biases observed was a consequence of dietary option indifference, failure to associate diet type with colour, failure to transfer knowledge in the previous phases to the new, novel colour pairings or an inability to distinguish easily between diets. To investigate these potential explanations the experimental design was modified.

3.7. Experimental modifications

3.7.1. Novel colour-pairing

3.7.1.1. Hypothesis

It was hypothesised that if the birds could transfer knowledge learnt in previous colour – pairings to novel colour – pairings then they would immediately prefer the food – rewarded option.

3.7.1.2. Method

Two novel colour pairs were created by switching the no food colours: the no food colour originally paired opposite the control diet colour was now paired opposite the experimental diet colour (and vice versa). The diet outcomes associated with each colour did not change. Birds were given 30 trials (6 days): ten per new colour pairing option and per Control versus Experimental diet option. Trials were blocked into groups of three. Each block contained one trial of each option. Within block trial order was randomised to reduce effects of current learning on performance (as opposed to choices reflecting previous learning).

3.7.2. Experimental diet versus control diet (2)

3.7.2.1. Hypothesis

It was assumed that if birds primarily attended to the 'no food' colours (i.e. they avoided the 'no food' option rather than specifically attended to whether "X" colour is associated with "X" diet option and "Y" colour is associated with "Y" diet option) then removal of this option would force attendance to the 'food' colours and result in discrimination between the two diet options (i.e. control diet and experimental diet). It was hypothesised that, in the continued absence of a 'no food' option, birds would learn to associate colours with diet quality and would show a preference for the more favourable option.

3.7.2.2. Method

Birds were given 35 trials (7 days) of the control diet versus the experimental diet options.

3.7.3. Experimental diet versus control diet + 50%

3.7.3.1. Hypothesis

It was hypothesised that if birds could learn to associate colours with differences in the properties (quality or quantity) then they would develop a preference for an option that provided more energy and nutrients.

3.7.3.2. Method

The control diet was increased by 50% to make it more attractive to hungry birds. Birds were given 55 trials (11 days) of the control diet versus the experimental diet options. Colours associated with each diet remained the same.

3.7.4. Two – pan choice test: experimental diet versus control diet

3.7.4.1. Hypothesis

Sensory diet discrimination is essential otherwise no choice is possible irrespective of how nutritionally diverse two diets are (Forbes and Kyriazakis, 1995). Although this had been previously tested in the pre – sensory phase, the lack of preference shown by CVF birds and the small preference shown by CVC birds potentially suggested that they have difficulties in discriminating between diets. Thus, despite how unlikely this may be, it was necessary to establish that the birds could distinguish between diets *per se*. It was hypothesised that, if the birds could discriminate between the two diets offered then they would prefer the control diet under simultaneous presentation with ad libitum access to both feeds.

3.7.4.2. Method

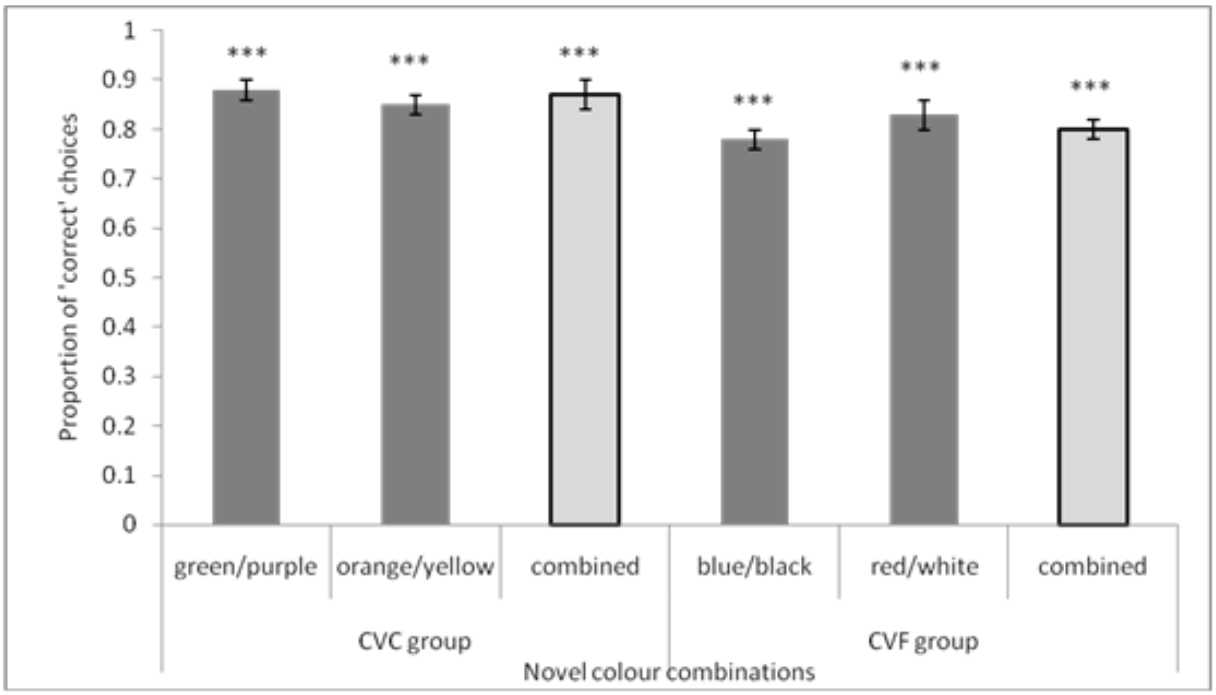
Birds were tested on the final study day and then humanely euthanised. Experimental apparatus was set up as in section 3.4.4.1. Each bowl was filled approximately ¾ full with either experimental or control diet which had been weighed. Within group, diet presentation was balanced for side (control diet was initially on left side for 50% of birds). At 0 min birds were placed into individual pens and allowed to freely consume from both bowls. At 10 min food was removed, weighed, replenished and returned to the pens (switched to the opposite side). At 20 min the procedure was repeated. At 30 min the food was removed, weighed and discarded.

3.8. Results (2)

3.8.1. Novel colour pairing

The results indicated that whatever the birds had learnt about the original training conditions they were able to transfer into the novel testing situation (CVF: $\chi^2 = 36.20$, d.f. = 1, $P < 0.001$; CVC: $\chi^2 = 46.49$, d.f. = 1, $P < 0.001$; Figure 4). There were no other significant effects or interactions. Individually, all birds achieved ≥ 15/20 ($p \leq 0.041$) choices for the colour associated with food.

Figure 4: Effect of novel colour combination on proportion of 'correct' (food –rewarded) options (see: section 3.7.1). Combined for each treatment represents the combined result of both colour-combinations within that treatment. *** = P <0.001. The error bars represent the S.E.M. associated with each combination.



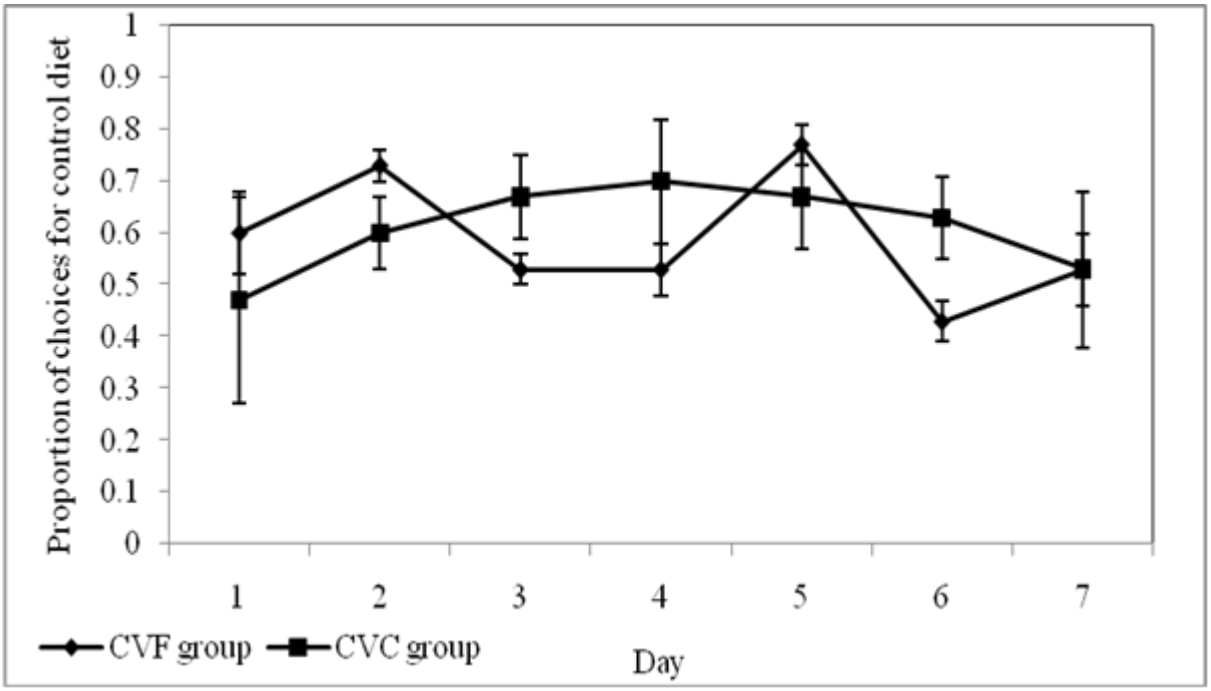
3.8.2. Experimental diet versus control diet (2)

3.8.2.1. Food preferences

As a group, birds did not exhibit a preference for either diet, either across all trials (CVC: $\chi^2 = 0.35$, $P > 0.1$; CVF: $\chi^2 = 0.23$, $P > 0.1$) or across days (Figure 5).There was no effect of treatment or day and no interaction between day and treatment. However, there was a highly significant effect of bird on the choices made ($W_{(11,3.31)} = 36.41$, $P < 0.001$). Post-hoc testing

indicated that two CVC birds and two CVF birds showed a significant preference for the control diet over the 35 trials. Four CVC birds and four CVF birds failed to show a diet preference.

Figure 5: Daily proportion of choices for either the control diet or the experimental diet by each treatment group (see: section 3.7.2.). 1 = 100% preference for control diet, 0 = 100% preference for experimental diet. There were no days on which a significant group preference for one of the diets (i.e. a significant difference from 0.5 choices for control diet) was shown. Error bars indicate the S.E.M. Figure legend: Closed diamond = CVF group; closed square = CVC group.



3.8.2.2. Side biases

None of the birds that showed a diet preference showed a side bias. Of the eight birds that did not show a diet preference, seven showed a significant ($P < 0.05$) side bias; the remaining bird tended ($P = 0.09$) to prefer one side over the 35 trials.

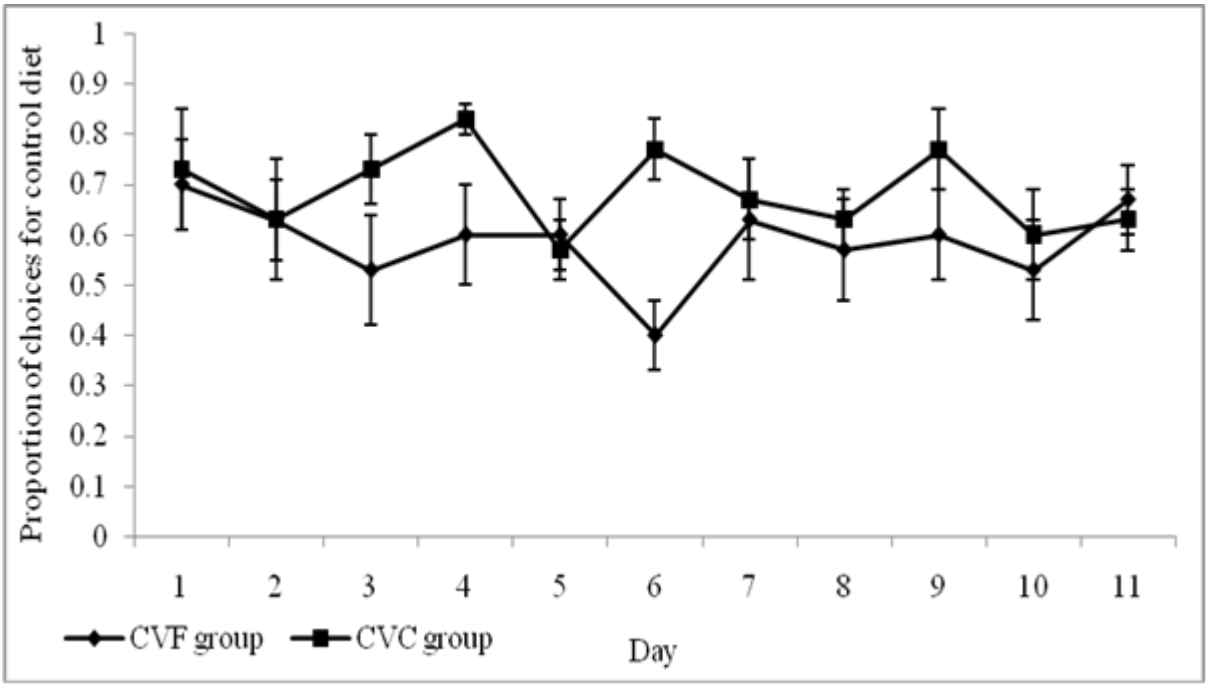
However, at the group level, there was no difference between the severity of side biases demonstrated in phase five (control versus experimental diet) and those exhibited in either the first or last ten trials of this phase (control versus experimental diet; $H = 2.136$, d.f. 2, $P = 0.328$).

3.8.3. Experimental diet versus control diet + 50%

3.8.3.1. Food preferences

Although neither treatment group showed an overall preference for either the experimental diet or the '50% extra' control diet (CVC: $\chi^2 = 0.01$, d.f. = 1, P > 0.1; CVF: $\chi^2 = 2.25$, d.f. = 1, P > 0.1), there were several days on which the CVC group selected the control diet significantly more often (see figure six). However, the 'performance' of both groups was sufficiently similar that significant differences between groups were not found either overall or by day, and there was no interaction between treatment group and day.

Figure 6: Proportion of choices for the control diet (50% extra) option each day by treatment group. There were no days on which the CVF group showed a significant preference. There were five days (day 1, 3, 4, 6, 9) on which the preference for the control diet was significant ($p < 0.05$) for the CVC group. Error bars indicate the S.E.M. Figure legend: Closed diamond = CVC group; closed square = CVC group.



However, there was a highly significant effect of bird on the choices made ($W_{(11,4.50)} = 49.50$, d.f. = 1, P < 0.001). Post – hoc testing indicated that three CVC birds and two CVF birds showed a highly significant preference for the control diet over 55 trials. A further CVC bird had a tendency to select the control diet and one CVF bird had a tendency to pick the fibre diet. The remaining five birds failed to show a diet preference, either over all 55 trials or over the last 15 trials.

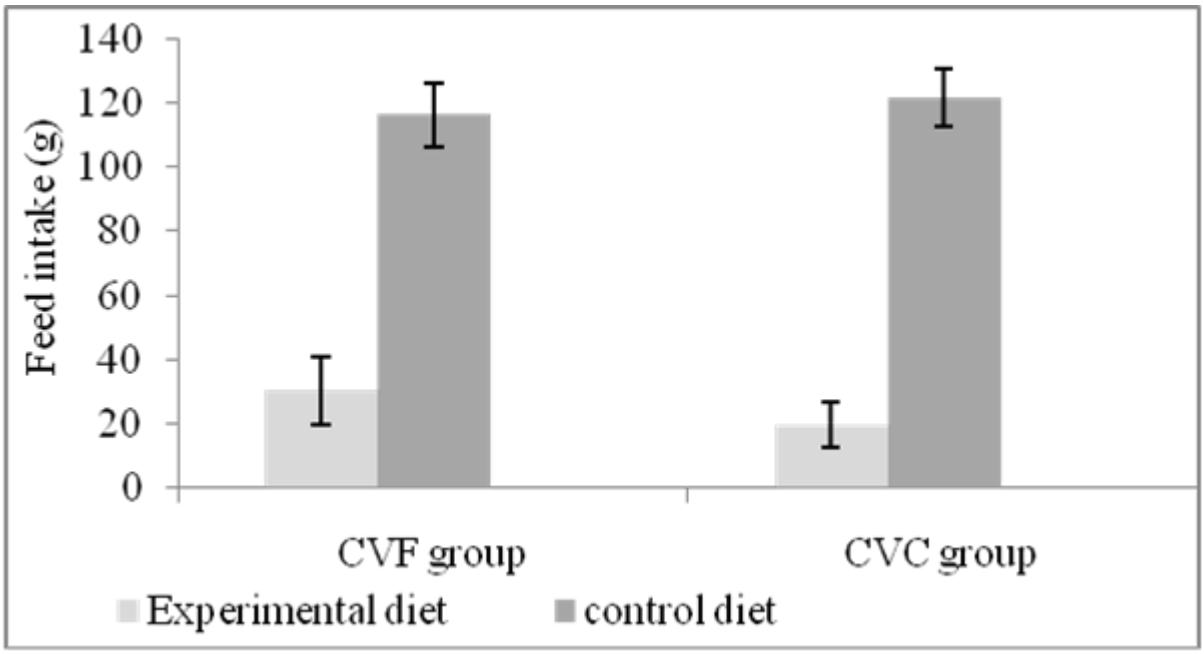
3.8.3.2. Side biases

Side biases remained prevalent. All birds that failed to show a significant diet preference (plus one CVC that did) demonstrated a side bias. Eight birds showed either a highly significant ($n = 4$, $P < 0.001$) or significant ($n = 2$, $P \leq 0.014$) side preference or had a tendency to pick one side more ($n = 2$, $P = 0.058$) over 55 trials.

3.8.4. Two-pan choice test: Experimental diet versus control diet

Overall, there was a highly significant effect of diet ($T = 1.0$, d.f. = 11, $p < 0.001$), with 11 out of 12 birds preferring the control diet. This demonstrated very clearly that the birds were able to distinguish between the two diets (Figure 7).

Figure 7: Mean consumption (grams) over 30 minutes of the experimental diet and the control diet. The error bars represent the S.E.M.



Within the CVF group, there was a significant preference for the control diet ($T = 0.0$, d.f. 5, $p = 0.031$) with all six birds preferring the control diet. Within the CVC group, there was a tendency to prefer the control diet ($T = 1.0$, d.f. 5, $p = 0.062$). However, this was probably due to the small sample size and lack of statistical test sensitivity (the non-parametric Wilcoxon Matched Pairs test was used for all three analyses), as five out of six CVC birds showed a strong preference for the control diet.

3.9. Discussion

3.9.1. Modifications

Clearly bird failure was not due to inability to transfer learnt information to solving a novel task or to distinguish between diets. Therefore, the lack of diet preference observed under the T – maze choice test conditions seemed due to difficulties associating diet quality and quantity differences with different colour maze arms. Reasons for this are discussed below.

3.9.2. Observed diet preferences

The birds strongly preferred the control diet under *ad libitum* conditions. High energy – density diets are often highly preferred (Bolles, *et al., 1981;* Brunstrom and Mitchell, 2007; Bouvarel *et al*., 2009). Utilising a similar two – pan, *ad libitum* access, choice test, Guillemet, *et al*., (2007) found gestating sows (highly food motivated) also prefer high quality nutrient dense feed to quality – adjusted, high fibre feed. Preference for nutrient – dense diets makes evolutionary sense: animals need to balance feed intake against other needs (for example, reproduction, predation avoidance, etc) (Illius, *et al.*, 2002 Lieberman, 2006*)*. Therefore, the direction of the preference observed was unsurprising.

Where significant preferences developed under closed economy, feed restricted conditions (prior to increasing control diet quantity) as they did for two CVC and two CVF birds, these preferences were also for the high quality, nutrient dense control diet. D'Eath, *et al.* (2009) suggests animals' preference for high quality feed over low quality feed might disappear under restricted feed conditions if the low quality feed confers improved satiety. Our results did not support this. However, we cannot rule out whether this was due to the experimental diets not having increased satiating effects (therefore not addressing the point) or impulsivity influencing choice by biasing any preferences towards the most rapidly consumable diet. Abeyesinghe, *et al*. (2005) found that chickens showed self-control only between a small immediate reward and a delayed (much) larger reward. This implied a need for the experimental diet to be much more rewarding if it is to be preferred. Although there was no time delay imposed on diet access, the experimental diets would take longer to consume compared to the control due to diet bulkiness (FIBRE) or additive fineness (CAP) (intake rate not measured). Anecdotal observation (unfortunately this was not formally measured)

indicated that the latency to fully consume either experimental ration rapidly decreased. However, this reduction may have been concurrent with a gradual decrease in satiating capacity due to physiological adaptation to the additional dietary components (Tolkamp *et al*., 2005) further reducing its additional 'rewarding' features over the control diet. Alternatively, it is possible that the use of a schedule in which the birds alternated between the control and experimental diets created a situation in which, even if the experimental diets had increased effects on satiety, because the birds were not maintained continuously on the experimental diet, the full satiation effects of these quality – adjusted diets were not achieved. Thus, the birds tested may not have been in the same physiological and / or affective state as birds reared continuously under conditions of qualitative feed restriction and this may have impacted upon both their ability to learn the task and / or to express any preference learnt.

In addition, group feeding species (including chickens) already eat faster than solitary feeders (Sunday, 1981, quoted in (Ackroff, 2002) and chickens have been shown to have a greater motivational drive to feed fast under chronic than acute feed restriction (Savory, *et al.*, 1993). Thus, the combined effect of species – specific characteristics and strong motivational drive may increase preference for rapidly consumable high quality feed, irrespective of possible later differences in diet – induced satiation. However, the design of the study may also have affected the presence or absence of preferences observed as the birds may never have experienced the degree of satiety that being reared entirely on a qualitatively restricted diet may offer. It remains a problem for choice test methodologies of this nature: the birds are inevitably reared, trained and tested under conditions that are not similar to commercial environments. However, as the current methodologies utilising environments close to those experienced under commercial conditions also fail to provide convincing evidence of the benefits or otherwise of qualitatively restricted diets these alternative approaches should be explored.

3.9.3. Methodological issues

A long inter-trial interval (ITI) ensured birds experienced the 'satiating' effects of their choice through the mechanism of post-ingestion feedback. Matthews and Temple (1979), used an operant choice test to allow dairy cows to access small quantities (time restricted access ~ 5 seconds, ITI variable interval 60 – 300 seconds) of either concentrate or hay. The authors claimed this allowed diet preference quantification without the confounding variable of post-ingestion effects. However, this can be a limitation. Post-ingestion feedback shapes

longer-term diet preferences (Forbes, 1998; Kyriazakis *et al*., 1999). Thus, we wanted a longer ITI with larger portions / trial.

However, it is possible the ITI was too long (90 min) for colour – diet information retention. Our birds easily learnt the food / no food task. Direct comparisons between speed of learning this task and the quality / quantity discrimination tasks are not methodologically possible. However, rats performed better with spaced trials than with massed trials (Sarason *et al*., 1956) but the ITI used in that case was only 12 minutes long. Pennington & Thompson (1958) found the number of trials needed for rats to reach the criterion increased with ITI length (ITI lengths compared: 40 min – 24 hours). However, other studies found a positive, negative or no effect of ITI on learning (D'Amato, 1960).

Failure to learn could also be attributed to decreased differential in terms of comparative option payoffs which increased task complexity. Rats learnt food – no food discrimination tasks more quickly than food quantity discrimination tasks (Clayton, 1964). Further, rat (Hill and Spear, 1963; Clayton, 1964) and dolphin (Mitchell *et al*., 1985b) acquisition rates are a function of the contrast between two reward quantities. We could not find any papers investigating feed quality effects on acquisition rate in similar choice test apparatus. However, non – feed restricted layer hens quickly associated diets with colours in a heavy – metal feed contamination versus no contaminated discrimination task (Phillips and Strojan, 2007). Although we cannot discard methodological reasons causing or contributing to the failure of most birds to learn the food quality and quantity discrimination tasks, the success of some birds indicated the task was potentially learnable. Thus, we were led to consider the internal physiological and affective state of the birds as a potential causal factor.

3.9.4. Hunger and stress

Hunger – stress may have decreased the birds' learning ability. Although motivation to gain feed increases with degree of feed restriction (Savory *et al*., 1993; Bokkers *et al*., 2004), hunger is also a stressor (Mendl, 1999).The Yerkes – Dodson model (Yerkes and Dodson, 1908) suggests there is a bell curve effect to arousal with an optimal level of arousal for effective learning. Although the model is simplistic (Mendl, 1999) a useful basic interpretive framework is provided by it. The birds' success at learning the food – no food tasks but failure to learn the food quantity / quality choice tasks corresponded with increasing severity of feed restriction. More complex tasks have a lower optimal arousal level (Yerkes and Dodson, 1908). Stress can reinforce inflexible, habitual learning (Mitchell *et al*., 1985a)

leading to poorer performance. Therefore, poor learning may have been the combined effect of being too hungry and the dietary option contrast being too small.

3.9.5. Side biases and stress

Although side biases may be an artefact of study design as chickens show low levels of spontaneous alternation in T – mazes (Haskell *et al*., 1998), we found that side biases increased with the change from the food / no food to food quality discrimination tasks. Feed restriction severity was also increasing throughout this study. Side biases are more prevalent in hunger – stressed starlings (Talling *et al*., 2002) and electric – shock stressed rats (Rodriguez *et al*., 1992). These preferences can manifest as increased perseverance (Rodriguez *et al*., 1992). Further, feed – restricted pigs in a food – no food T – maze task showed side biases even when they could see food in the non – selected pen (Rodriguez *et al*., 1992). Reducing pig arousal by reducing time in the start box improved performance (pigs picked the food option). These findings suggest species – specific tendencies reinforced by the effects of stress may have affected T – maze performance in our study.

3.10. Conclusions

In conclusion, the selected T – maze task was not useful in investigating the feed preferences of chronically feed restricted broiler breeders. Although where birds did learn the task they preferred a small quantity of high quality feed to a quality – adjusted diet, the small number that did so limit any firm conclusions. Nevertheless, should a larger sample size replicate this preference, this would be an interesting avenue for further investigation. However, preference exploration is based on the implicit assumption that cognitive capacity to learn and exhibit a preference is not undermined by chronic hunger stress. This suggests that the impact of chronic hunger – stress on broiler breeder learning should be studied first in further investigative research focussing on feed restricted broiler breeder dietary preferences.

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4. Experiment two: Too hungry to learn? Hungry broiler breeders fail to learn a T-maze food quantity discrimination task

4.1. Preamble

This experimental chapter was published in the peer-reviewed journal Animal Welfare. The relevant reference is:

Buckley, L. A., McMillan, L. M., Sandilands, V., Tolkamp, B. J., Hocking, P. M. and D'Eath, R. B. (2011) Too hungry to learn? Broiler breeders fail to learn a T-maze food quantity discrimination task. *Animal Welfare* 20: 469 – 481

Aspects of this study have also been presented at two external conferences. The relevant references are:

Buckley, L. A., McMillan, L. M., Sandilands, V., Tolkamp, B. J., Hocking, P. M. and D'Eath, R. B. (2011) Hungry broiler breeders fail to learn a food quantity T – maze discrimination task. *World Poultry Science Association (UK branch) Annual Meeting*, 13th – 14th April, Belfast, UK

Buckley, L. A., McMillan, L. M., Sandilands, V., Tolkamp, B. J., Hocking, P. M. and D'Eath, R. B. (2011) How hungry is too hungry? Impaired learning leads to poorer payoffs in hungry broiler breeders. *Recent Advances in Animal Welfare Science (Universities Federation for Animal Welfare Animal Welfare Conference)*, 21st June, York, UK

I was ably assisted on this project by a University Of Edinburgh student, Mrs Lisa McMillan, who was undertaking the research component of her MSc in Applied Animal Behaviour and welfare. Lisa's experiment was concerned with the running speed and behavioural observations of the broiler breeders whilst in the Y – maze arms. Her contribution to this experiment included assisting with choice test data collection, primary management of the video equipment, video-watching and recording of the bird latencies to exit the Y – maze apparatus along with some of the general day – to – day husbandry of the birds. The data was re-analysed by the author for the purpose of this experimental chapter and paper publication.

4.2. Abstract

Choice tests may aid the determination of whether qualitative dietary restriction improves the welfare of feed restricted broiler breeders. However, hunger – stress may reduce competency to choose by impairing learning. The effect of chronic feed restriction on the ability of broiler breeders to learn a hunger – relevant discrimination task was investigated using a Y – maze paradigm. The task was to associate black and white arms with large and small quantities of feed. Birds were reared to three growth curves by means of severe (n=12), moderate (n=12) or very mild feed restriction (n=12). Learning the task and selecting the larger food option allowed birds to increase their feed intake. Time taken to traverse the Y – maze was also measured.

Birds from all treatment groups traversed the Y – maze more quickly over time indicating that they had learnt that running down the Y – maze arms was associated with a rewarding outcome (food). However, feed restriction significantly reduced their ability to associate the black and white cues with differences in food quantity. Consequently, average payoffs in terms of daily feed increments disproportionately accrued to the less feed restricted treatment groups.

It is concluded that feed restriction affected the performance of broiler breeders in this task, perhaps by narrowing their attention such that they ignore potentially hunger-relevant contextual cues. However, low overall group success rates demonstrate that this task was difficult to learn even for less severely feed restricted birds. Therefore, Y – maze choice tests may not be the most appropriate method for determining hungry broiler breeder dietary preferences.

4.3. Introduction

Hunger is the most pressing welfare issue facing the modern-day broiler breeder. Selective breeding for large appetites facilitates rapid growth in birds destined for consumption but also results in parent stock that must be feed restricted to ensure optimal growth rates. *Ad libitum* feeding regimes are associated with obesity and co-morbid conditions such as ascites syndrome (Baghbanzadeh and Ducuypere, 2008), increased lameness (Kestin *et al.*, 2001) and reproductive failure (Robinson & Wilson, 1996). Thus, is it is necessary to feed restrict broiler breeders to 25 – 50% of *ad libitum* intake (Savory & Maros, 1993). However, this results in a bird that experiences chronic hunger for most of its life. By six weeks of age, broiler breeders consume their daily ration within 5 - 7 minutes (Savory & Maros, 1993), show various behavioural and physiological indicators of stress (Hocking *et al.*, 1993; Hocking *et al.*, 1996; de Jong *et al.*, 2002; de Jong *et al.*, 2003) and are prepared to work for additional feed even when reared on double the recommended ration of feed (Savory *et al.*, 1993).

A popular scientific approach has been to try to improve satiety by modifying the quality of the feed ration. Low – or non – nutritive fillers such as ground oat hulls and / or appetite suppressants (e.g. Zuidhof *et al*., 1995; Savory *et al*., 1996; Rozenboim *et al*., 1999; Vermaut *et al*., 1999; Savory and Lariviere, 2000; Nielsen *et al*., 2003; Hocking *et al*., 2004; Sandilands *et al.*,2006; Hocking, 2006) are added to the ration to try and increase satiety without increasing energy intake. However, the evidence that this improves welfare in broiler breeders is unclear and variable (see: Savory *et al.*,1996; Savory and Lariviere, 2000; Nielsen et al., 2003; de Jong *et al*., 2005; Sandilands *et al*., 2005; Sandilands *et al*., 2006; Hocking, 2006). Therefore, there is a need for additional methods. D'Eath, *et al.* (2009) suggested that choice tests could be a valuable additional tool to enable us to identify whether broiler breeders prefer traditional, quantitative dietary restriction or qualitative dietary restriction. Buckley *et al.* (2010) used a T – maze closed economy choice test task to determine hungry broiler breeder preferences for quantitative or qualitative dietary restriction. They found that whilst birds easily learnt a food versus no food discrimination task irrespective of the food type offered, the birds failed to show a preference for either diet in a similar food quality discrimination task. However, the same birds largely failed to learn a food quantity discrimination task. Thus, the authors concluded that the failure to show a preference was

indicative of a failure to learn the discrimination task and not a lack of dietary preference *per se*.

One possible explanation for the findings of Buckley *et al.* (2010) is that the birds were too stressed or aroused by hunger and that this negatively affected their ability to learn the more complex quantity and quality discrimination tasks. The interaction between stress (whether acute, chronic or both are present) and learning is complex. Experimentally – induced chronic stress was found to negatively affect acquisition of food – rewarded cognitive spatial tasks in 75% of the studies examined by Conrad (2010). Nicol and Pope (1993) found that short term feed restriction reduces social learning in hens which may have implications for any hen social preferences observed. If acute stress is experienced during testing this may also affect cognition by affecting the ability to learn or remember the key features necessary to make an informed choice (Mendl, 1999). However, rats in a Morris water maze performed better when tested in cold, rather than warm, water (Sandi *et al.*, 1997) suggesting that there is a positive relationship between performance and stressor severity when the task is relevant to removal or reduction of the stressor.

This has implications for feed restriction as a stressor in food – rewarded choice tests that have a strong discriminative and associative aspect to the study design. Hunger is considered to be both a negative stressor (Dawkins. 1990; D'Eath *et al.*, 2009) and a positive motivator (Diano *et al.*, 2006). Feed choice tests for hungry broilers are stressor – relevant. Using chronic feed restriction (to maintain 95, 85 and 75% of *ad libitum* bodyweight), Richman *et al.* (1970) observed that rats learnt different two similar food versus no – food T – maze tasks faster the greater the difference between their actual and *ad libitum* bodyweight. This suggests that hungry animals would learn a food rewarded discrimination task more quickly than a sated one. In previous work (Buckley *et al.*, 2010) we found that chronically food – deprived broiler breeders easily learnt a food / no food T – maze task, but most failed to learn a task in which both options were rewarded but with different food quantities. However, discriminative tasks in which both options are rewarded are generally considered to be more difficult to learn than reward / no reward tasks (Capaldi & Molina, 1979). Research by Yerkes and Dodson (1908) indicates that the more difficult the task, the lower the arousal level that is required for optimal learning. Thus, direct choice tests where the broiler breeder has to learn a feed quality discrimination task may fail if the bird is trained and tested under the conditions of feed restriction in which such a preference could be welfare – relevant.

Therefore, there is a need to identify the effects of feed restriction on broiler breeder ability to learn a feed discrimination task in which both options are differentially rewarded.

The present study investigated the ability of broiler breeder chickens (*Gallus gallus domesticus*) reared on three different levels of feed restriction to learn a complex feed quantity discrimination task. It was assumed that birds would prefer a larger over a smaller quantity of feed, which means that 'success' can be measured as number of choices for the larger feed reward. It was hypothesised that the degree of feed restriction – induced arousal will alter task learning ability and the maintenance of the learnt response. It was predicted that there would be a negative relationship between the degree of feed restriction and bird performance in the discrimination task due to its complexity. The latency of birds to make a choice was also measured. It was predicted that birds would become faster at making a choice as they learnt to associate the ends of the Y – maze arms with the presence of feed.

4.4. Methodology

4.4.1. Subjects

Fifty-two Ross 308 broiler breeders were obtained as day old chicks. At 14 days, the birds were ranked, blocked according to weight then and randomly allocated to a treatment (level of feed restriction). The four treatment groups were: *Ad libitum* (n=16), Eighty% (n=12), Forty% (n=12) and Control (n=12). The *Ad libitum* birds were used only to establish *ad libitum* intake and did not take part in the choice test training / testing.

4.4.2. Housing and husbandry

Birds were spot-brooded in five groups (n=16 each) until day 14 in 1 m x 1 m pens containing wood shavings, a perch and a drinker allowing *ad libitum* access to water. From day 14 birds were individually housed (two rooms, 26 pens in each room, with treatments equally distributed across both rooms) in pens of dimensions 0.5 m x 1.0 m with visual access to another bird (from the same treatment to reduce stress that could result from differential bird size or access to feed). Producer recommendations for lighting/heating were followed with a gradual reduction in light hours (from 23h on day 1 to 8h on day 10) and heating (from 31˚C on day 1 to 21^0C on day 21) (Aviagen, 2006). However, shed temperatures frequently exceeded recommendations during the last few study weeks due to external ambient temperature (Average (±std. deviation) / maximum / minimum temperature: Room 1: 23.4˚C (± 2.1) / 33˚C / 19˚C; Room 2: 22.1˚C (± 1.8) / 32˚C / 19˚C).

4.4.3. Nutrition and feeding

4.4.3.1. Feed type

Birds were fed a standard broiler starter crumb (Laser SP starter Crumb, BOCM Pauls Ltd, Ipswich, Suffolk) containing 205g crude protein (CP)/kg and 12.5MJ ME/kg until day 28. They were then switched to a custom-made grower mash diet (Target Feeds Ltd, Whitchurch, Shropshire) containing 150g CP/kg and 11.5MJ ME/kg. After switching, poor diet acceptance rapidly reduced growth rate and increased the within-group bodyweight coefficient of variance (CV). The reason for this poor acceptance was unknown but was likely to relate to

an aspect of diet quality. Consequently, birds were switched back to the original diet on day 33 and fed this for the rest of the experiment.

4.4.3.2. Feeding regime (day 1 – 41)

All birds were fed *ad libitum* during the first week and a restricted allowance during the second week according to producer recommendations (Aviagen, 2007). From 14 days, birds were fed according to their treatment protocol. *Ad libitum* birds were fed *ad libitum* for the study duration. Feed intake was measured once daily between 08:00h – 08:45h via a weigh back technique to ascertain feed intake for the previous 24h period. For all other treatment groups, birds were fed once daily at 08:45h – 09:15h until day 42. All birds were individually weighed daily at 08:00h – 09:00h (before being fed) until day 49 and twice weekly thereafter. The Control group were fed to maintain them on the recommended growth curve for Ross 308 Broiler Breeders reared to have 5% egg production at 25 weeks of age (Aviagen, 2007) with quantities adjusted as necessary. This is the most common rearing strategy adopted worldwide by producers of Ross 308 parent stock. The average feed intake per bird per day was calculated daily for the *Ad libitum* and Control birds and used to calculate feed allowance for birds in the Forty% and Eighty% groups. Therefore, the Control group experienced the most severe level of feed restriction, the Eighty% group the least severe and the Forty% group were intermediate between these two groups.

Individual birds in the Forty% and Eighty% groups received an allowance according to the following formula:

Allowance = mean control intake + (P × (mean *ad libitum* intake – mean control intake)

Mean intake refers to intakes recorded on the previous day and the P values were 0.4 and 0.8 for the Forty% and Eighty% treatments respectively.

This formula was used instead of using the more simple approach of either calculating feed intake for the different feed restricted treatment groups as a proportion of *ad libitum* intake or as a multiple of the commercial quantity of feed restriction. The rationale for adopting this

approach was to ensure that the relative degree of feed restriction was constant between groups at all times.

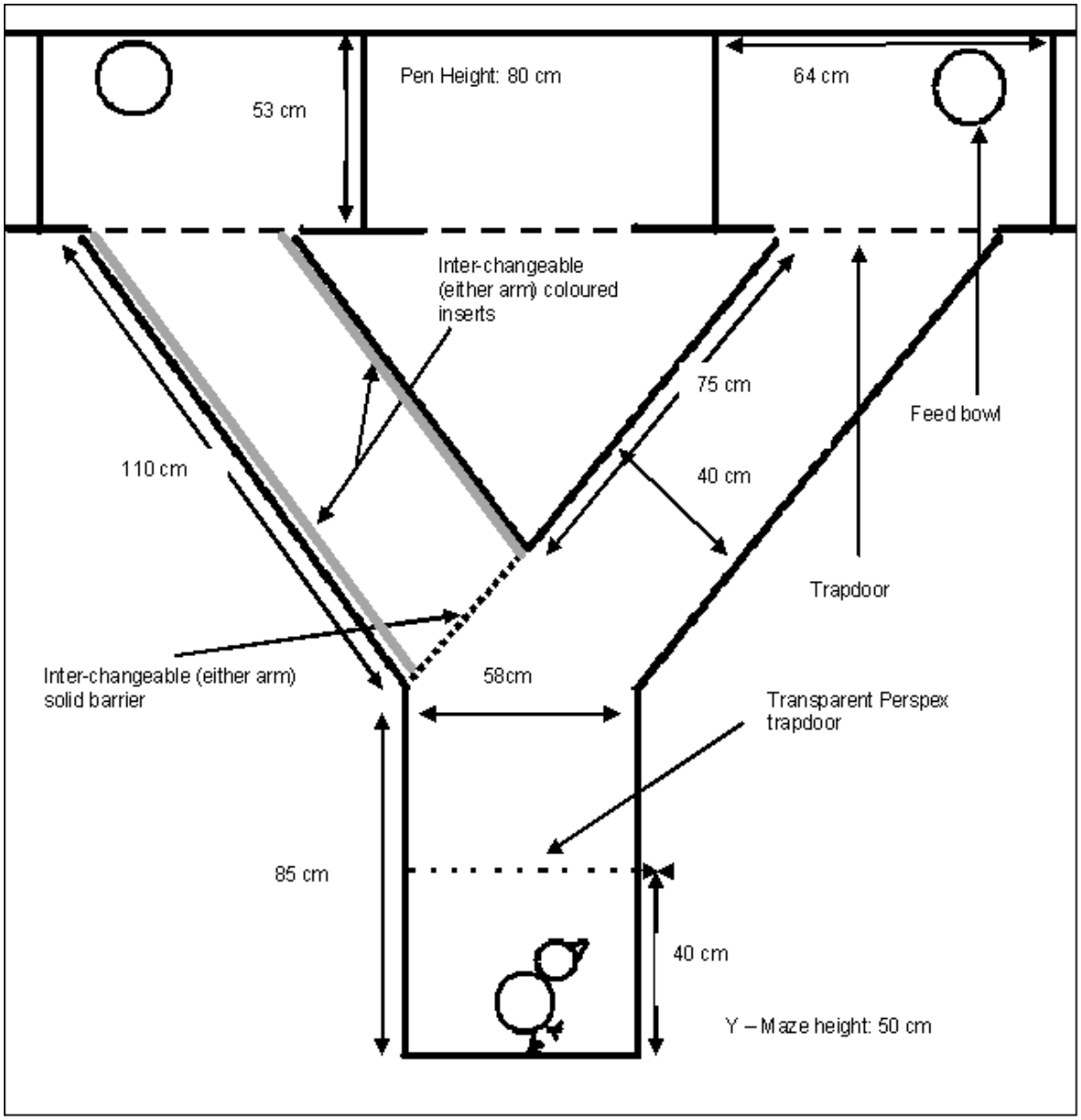
4.4.3.3. Feeding regime (day 42 – day 72)

Between days 42 and 48, the Control, Forty% and Eighty% birds were fed 1/5 of the Control birds' daily ration 5 times / day at 75 minute intervals in preparation for training and testing. The Forty% and Eighty% birds were fed the remainder of their feed allowance at the end of the day. Unconsumed feed was removed between 20:00h and 22:00h and added to the same bird's end of day ration the following day. This was only occasionally necessary for some Eighty% birds and was never needed for the Forty% birds. This feed removal protocol was maintained until the end of the study.

4.4.4. Experimental apparatus

Both rooms housed an identical experimental set up. The experimental apparatus was comprised of a plywood Y – maze (see: figure 1 for dimensions) and 12 wooden goal boxes (pens containing a food bowl) with manually operated trap doors. The food bowls were circular (diameter: 18cm, height 6cm, volume: 0.5L) allowing immediate visual assessment of the quantity of feed contained by the relative amount of the base of the bowl that was covered by feed. The Y – maze was mounted on castors to make it easy to move, allowing rapid sequential testing of several birds. The arms lined up with the goal box openings. Each bird had access to the same two goal boxes during each free choice trial. The start pen had an opaque roof and a clear Perspex trapdoor. Removable painted wooden inserts fitted the insides of both arms, which allowed either arm to be coloured black or white. A camera was attached by a rod to the back of the start box and angled to allow all activity that occurred in the Y – maze (excluding the start box) to be observed. The camera was connected by cable to a DVR system (Xvision, Croydon, Surrey) that was remotely located in a separate room.

Figure 1: Experimental set-up showing Y – maze and test pen (goal box) dimensions.
Please note: drawing is not drawn to scale.



4.4.5. Testing procedure

4.4.5.1.General procedure

The task was to associate the black and white arms of the Y – maze with large and small food rewards (or vice versa). The black arm was associated with the larger reward for half of the birds (selected at random within treatment) and with the smaller reward for the remainder. The feed rewards were supplied within the goal boxes (one reward per goal box, with the feed supplied in identical bowls).

The two dietary rewards were 1/5 of the Control bird's daily ration + or – 25% (12 g versus 7.2 g, average payoff if no preference was shown: 9.6 g /trial). These quantities were modified after the 20 forced trials (4 days) and 60 free choice trials (12 days) had been completed to 1/5 of the Control bird's ration at 67 days + or – 50% to increase the contrast between the two options and recruit additional 'successful' birds (15 g versus 5 g, average payoff if no preference was shown: 10 g / trial). Learning the colour-portion quantity association enabled birds to supplement their diets by an additional maximum of 12.5 g / day (trials 1 – 60) and 25 g / day (trials 61 – 100).

Each bird was given five trials / day (75 minutes apart). Birds were held in the start box for 30 seconds before release. Each trial ended with entry into one of the goal boxes, which was then closed and the bird given approximately twenty minutes to consume the ration. The end – of – day ration was unaffected by individual performance during trials.

Any of the feed in the goal box that was chosen by the bird but not consumed during each trial, irrespective of whether the larger or smaller option was selected, was added to the end – of – day ration of that individual bird. The feed associated with the option not chosen by the bird was discarded. Although this was not recorded systematically, it was noted that Control birds never left feed obtained on the daily trials, while birds in the Forty% group occasionally left a 'fine covering' and the Eighty% group frequently left substantially more (even when the portion of feed obtained was the smaller of the two possible options).

4.4.5.2.Test apparatus habituation

Days 42 – 48: Each bird was given five twenty minute exploratory sessions (once daily for five days) within the Y – maze to habituate them to the apparatus. Part of the birds' daily feed allowance was sprinkled throughout the maze to encourage exploration.

4.4.5.3.Forced-choice trials

Days 49 – 52: Birds in the treatment groups Control, Forty% and Eighty% were each given 10 white runway forced trials and 10 black runway forced trials over the four days. These were balanced to ensure that the birds experienced the black arm and the white arm on the right side of the maze 5 times and vice versa. The feed quantities associated with the black and white arms and the feeding protocol are outlined in the general procedure section above.

4.4.5.4.Free-choice trials

Days 53 – 72: Birds experienced 100 free trials in which the bird could choose which Y – maze arm to enter. All other conditions (including feed quality and availability) remained the same. Feed quantity and contrast was increased after 60 free trials.

4.4.5.5.Measurement of latency to enter a goal box

All trials (forced and free) were video – recorded for later analysis of latency to make a choice. Latency to make a choice was measured from the time point at which the Perspex barrier was lifted until the time point the bird's head reached the feed bowl located in the one of the two goal boxes (measured in seconds).

Six days of free choice footage were selected for analysis. These were days 53 and 54, days 61 and 62 and days 69 and 70, representing the start, middle and end of the test period. Latencies were recorded for all trials that occurred on these days. All latency measurements were carried out twice by the same person. Where a difference between recorded latency occurred (<10% of clips watched), the clip was viewed a third time and the mean of the two closest measurements was recorded. Where a difference was observed this was never more than 1 second.

4.4.6. Statistical analysis and blocking

4.4.6.1. Blocking

Treatments were balanced across the two rooms. Within room, birds (n = 18) were blocked into three groups (n = 6) (balanced for treatment / colour – diet quantity combination) and testing order initially randomised within block (this order was maintained for the study duration).

During free trials, within – bird trials were grouped into blocks of 20 consecutive trials. Within blocks, trials were balanced for colour / side presentation with order of presentation randomised within and between birds.

4.4.6.2. Success criterion

Individual birds were defined as having learnt the task if they choose the larger feed reward ≥ 15 out of 20 times per trial block ($p \leq 0.042$, individual binomial probability). In the

analysis of the effect of colour combination on task success, only data recorded in blocks during which birds had learnt the task were included.

4.4.6.3. Statistical analysis

Individual bird performance was analysed using Probability Distribution Calculations for Binomial data. The Generalised Linear Mixed Models (GLMM) statistical test (logit – transformed binomial distribution, Schall method) was used to analyse dietary treatment level performance and to generate logit – transformed predicted means (group means per phase and overall performance). The variable of interest was 'food option chosen' and the fixed effects investigated included treatment, phase (1 – 2), trial block (1 – 5), room, and colour associated with the larger food option. Bird was used as the random effect (with trial nested within bird). Phase one represented the first 60 free choice trials (trial blocks 1 – 3), phase two the last 40 trials (trial blocks 4 – 5) and overall performance all 100 free choice trials (trial blocks 1 – 5). The statistical package used was Genstat version 11.1 (VSN International Ltd., Hemel Hempstead, UK).

Using the logit – transformed predicted means, post – hoc group analyses of differences from 0.5 were calculated using χ^2 to compare for differences from 0 at 1 degree of freedom using a Chi-squared (χ^2) – distribution table (Petrie & Watson, 1999). Due to the unplanned removal of one Eighty% bird after 60 trials, 'success' data was transformed from number of birds to proportion of birds that met the individual success criterion / treatment group before graphical representation.

A Linear Mixed Model was used to investigate effects of treatment on latencies and comparisons between latencies at the start, middle and end. Within group tests to compare changes between latency at the beginning and end of the study were performed using the Matched-Pairs Student t – test. Two birds in the Eighty% group were excluded from the latency analysis due to either incompleteness of data (one bird) or aberrant behaviour (latency increased during the experiment, probably due to leg problems).

4.4.7. Ethical considerations

This study was carried out under the Animals (Scientific Procedures) Act 1986 and approved by the Scottish Agricultural College's and Roslin Institute's ethics committees. The Home Office Code of Recommendations for the housing of poultry was met or exceeded at

all times. Birds were euthanised by an approved Schedule One method (barbiturate anaesthetic overdose). The relevant predetermined humane – end points used in this study were as follows: (i) birds weighing less than 90% of the target commercial weight at any stage were to be fed supplementary feed and any that failed to gain sufficient weight by supplementary feeding were to be euthanised (no birds were euthanised on this basis); (ii) although this study was designed to finish before birds would reach high bodyweights and associated problems such as lameness and respiratory problems, birds showing signs of such problems were to be removed from the study and euthanized. Overall, three birds were euthanized early under the banner of this humane end – point: one bird (Eighty% group) was removed due to lameness, two birds (*Ad libitum* group) due to lameness and the study was terminated 4 days early (day 72) because of thermal discomfort related to high room temperatures

4.5. Results

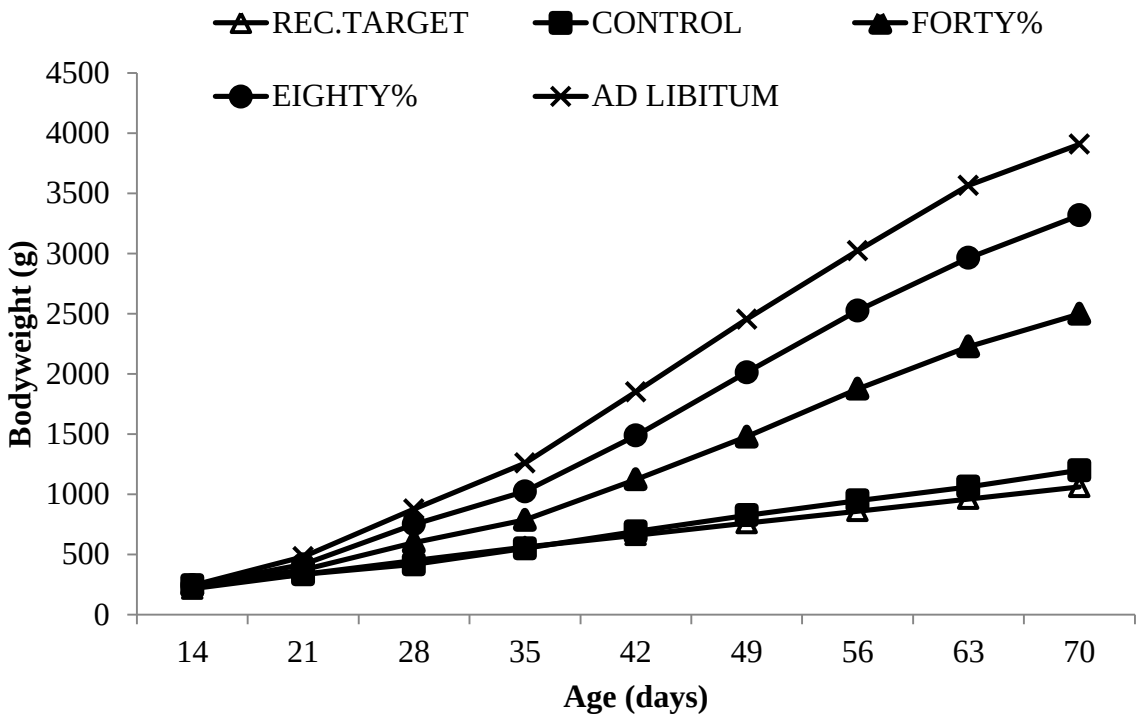
4.5.1. *Treatment growth curve and feed intake*

The growth curve of the control birds was similar to that of the producer's recommended target growth rate up to the start of the trial at 49 days of age (Figure 2). Birds on all treatments were successfully reared to their target growth curves, with only a small amount of variation. The mean (± s.e.m) coefficient of variation (CV) across time points and standard deviation associated with each treatment group was: *Ad libitum* group: 5.2% ± 0.9; Control group: 4.7% ± 1.5; Forty% group: 3.8% ± 1.3; Eighty% group: 5.0% ± 1.7. No overlap in body weights between groups was observed between days 21 – 70. This CV was small and well within producer recommended standards (Aim: < 12%; Aviagen, 2001).

The average daily intake of the *Ad libitum* birds increased from an average of 46.3g on day 14 to 235.1g on day 70. During this same period, the average daily feed allocation to the Control birds increased from 29g to 50g.

Figure 2: Bodyweight (g) by broiler breeder age and treatment group.

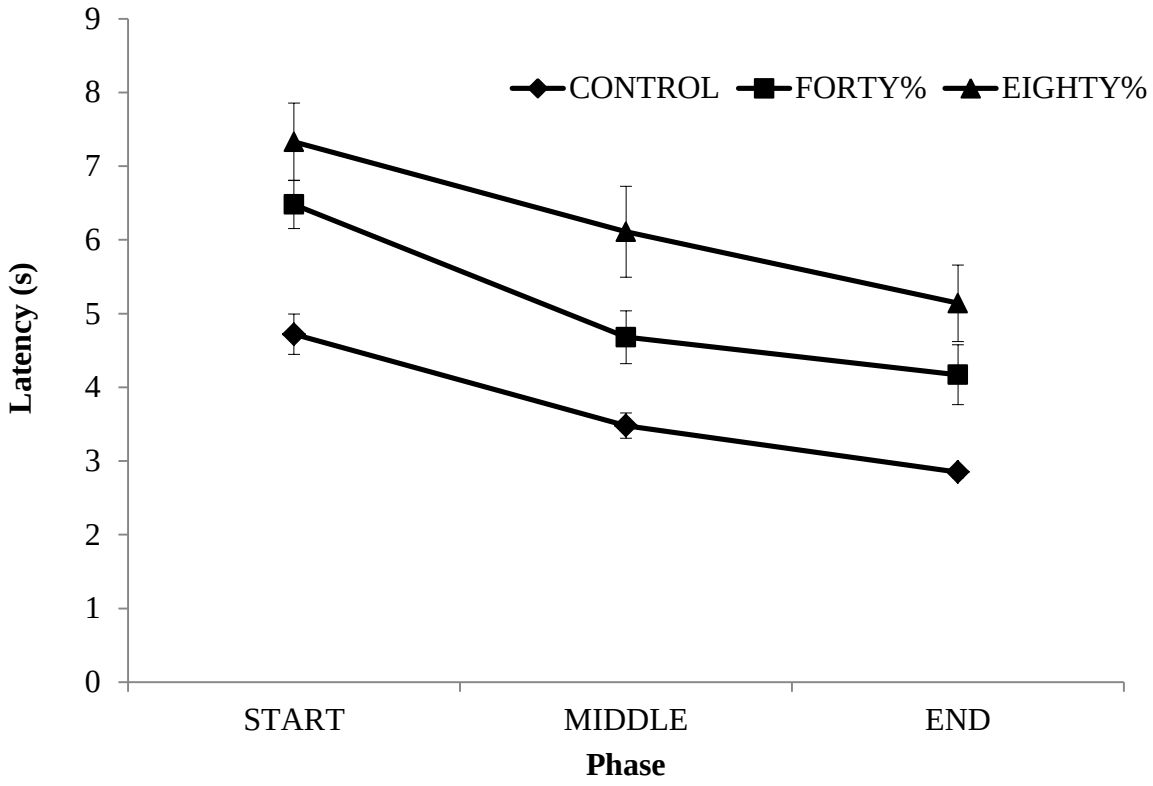
Rec. target represents the producer's recommended target growth rate for feed restricted broiler breeders grown to have 5% production at 25 weeks of age (shown for comparison). No error bars are shown as the S.E.M. was so small as to be graphically indiscernible. The average S.E.M for each treatment was as follows: *Ad libitum* group = 23 g; Control = 25.5 g; Eighty% group = 25.6 g; Forty% group = 25.5 g.



4.5.2. Effect of treatment and stage of testing on latency to enter a goal box

Treatment ($F_{2,31} = 12.84$, $P < 0.001$) and stage of testing (start/middle/end) ($F_{2,62} = 103.8$, $P < 0.001$) affected latency to enter a goal box but there was no interaction between treatment and stage of testing ($F_{4,62} = 1.13$, $P = 0.352$). Post-hoc testing using a series of paired Student's t – tests indicated that birds from all treatment groups ran faster as they learnt to associate the ends of the Y – maze with food (Eighty% group, $t_9 = 6.48$, $P < 0.001$; Forty% group, $t_{11} = 8.51$, $P < 0.001$; Control group, $t_{11} = 7.11$, $P < 0.001$). However, as can be seen from Figure 3, the more severe the feed restriction the faster the birds ran at all stages of testing.

Figure 3: Latency to enter goal box by treatment and stage of testing Nb. The error bars indicate the standard error. Two Eighty% birds were omitted from the analysis (see text).



4.5.3. Effect of treatment on ability to learn task

Overall, six Eighty% birds, three Forty% birds and one bird in the Control group were considered to have learnt the task. One Forty% bird and one Control bird achieved this criterion on the first or first and second free choice testing blocks but subsequently performed no better than chance for the remainder of the blocks.

A significant preference for the smaller reward option was never observed in any of the 180 individual blocks (36 birds x 5 blocks). Furthermore, only one Control bird was successful, in trial block one only, but two Forty % and five Eighty% birds (not including one successful Eighty% bird that was euthanised earlier in the study) were successful at the same time.

Analysis of group performance indicated that treatment affected the ability of the birds to learn the task ($F_{2,30.8} = 4.88$, $P = 0.014$). Post – hoc analysis revealed that, at the group level, only birds in the Eighty% group showed a preference for the larger feed option over the first sixty trials (Phase One)($\chi^2 = 8.16$, $P < 0.01$), the last forty trials (Phase Two) ($\chi^2 = 6.76$, $P <$

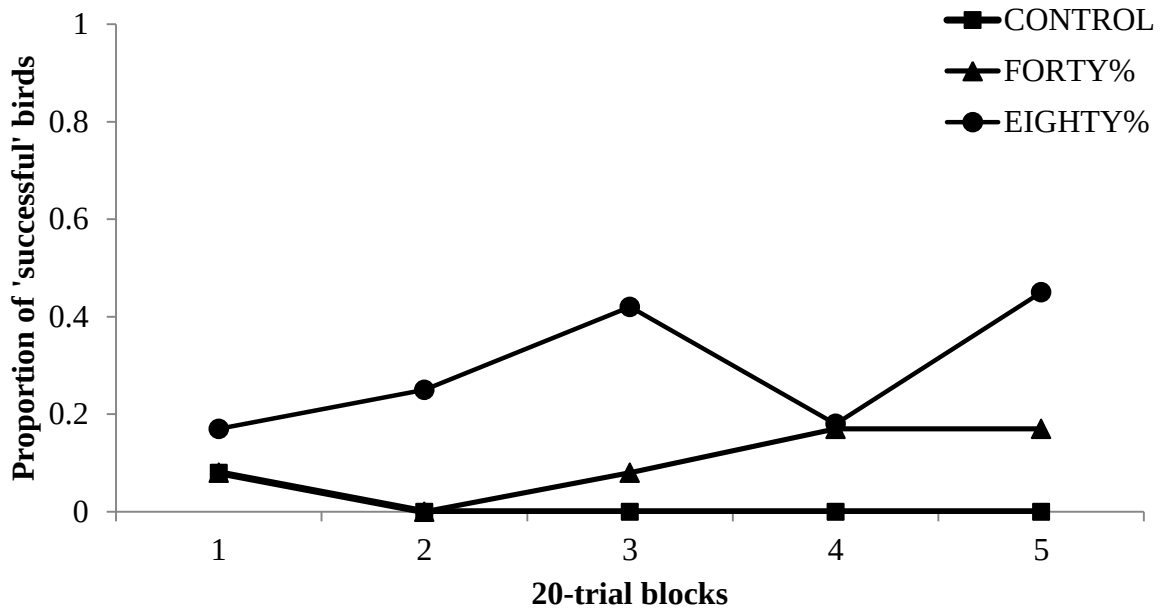
0.01) and across all trials ($\chi^2 = 8.60$, $P < 0.01$). The other treatment groups showed no preference. There were no significant effects of room ($F_{1,29.2} = 0.12$, $P = 0.729$) or colour associated with the larger food reward on the performance of successful birds ($F_{1,6.7} = 2.4$, $P = 0.167$).

4.5.4. Effect of level of feeding motivation on maintenance of response once learnt

Using all bird data, there was no effect of number of free choice trials (analysed in blocks of twenty consecutive trials) on performance ($F_{4,279.2} = 0.43$, $P = 0.788$) and no interaction between treatment and block number ($F_{8,348.6} = 0.60$, $P = 0.775$) with the average treatment performance remaining similar across all blocks of twenty trials. The numbers of 'successful' birds were too low to repeat the analysis using only these birds. Figure 4 shows the proportion of birds in each treatment group that were successful over each of the five consecutive blocks.

Figure 4: The proportion of birds by treatment and block achieving ≥ 15/20 choices for the larger food option (i.e. 'successful' birds).

A score of 15/20 was associated with $P = 0.042$ (binomial probability). n = 36; n = 12 per treatment; for Eighty% n = 11 for blocks 4 – 5 as one bird was euthanised.



Due to a lack of sufficient individuals within each treatment group meeting the task criterion for success, it was not possible to investigate the effect of feeding motivation on maintenance of the learnt response.

4.5.5. Effects of Phase One and Phase Two on performance

A comparison between group (i.e. analysed at the treatment group level) bird performance in Phase One and Phase Two demonstrated that there was no significant increase in performance across these two phases ($F_{1,91.7} = 0.11$, $P = 0.737$) and no interaction between phase and treatment group ($F_{2,92.2} = 0.09$, $P = 0.910$).

One additional Forty% bird achieved the inclusion criteria during Phase Two in which there was a greater contrast between the feed rewards. Three of the six 'successful' Eighty% birds showed a temporary decline in performance during the first 20-trial block in Phase Two but all three birds showed a significant preference ($\geq$ 15/20 choices for the larger feed reward) by the second 20-trial block in Phase Two.

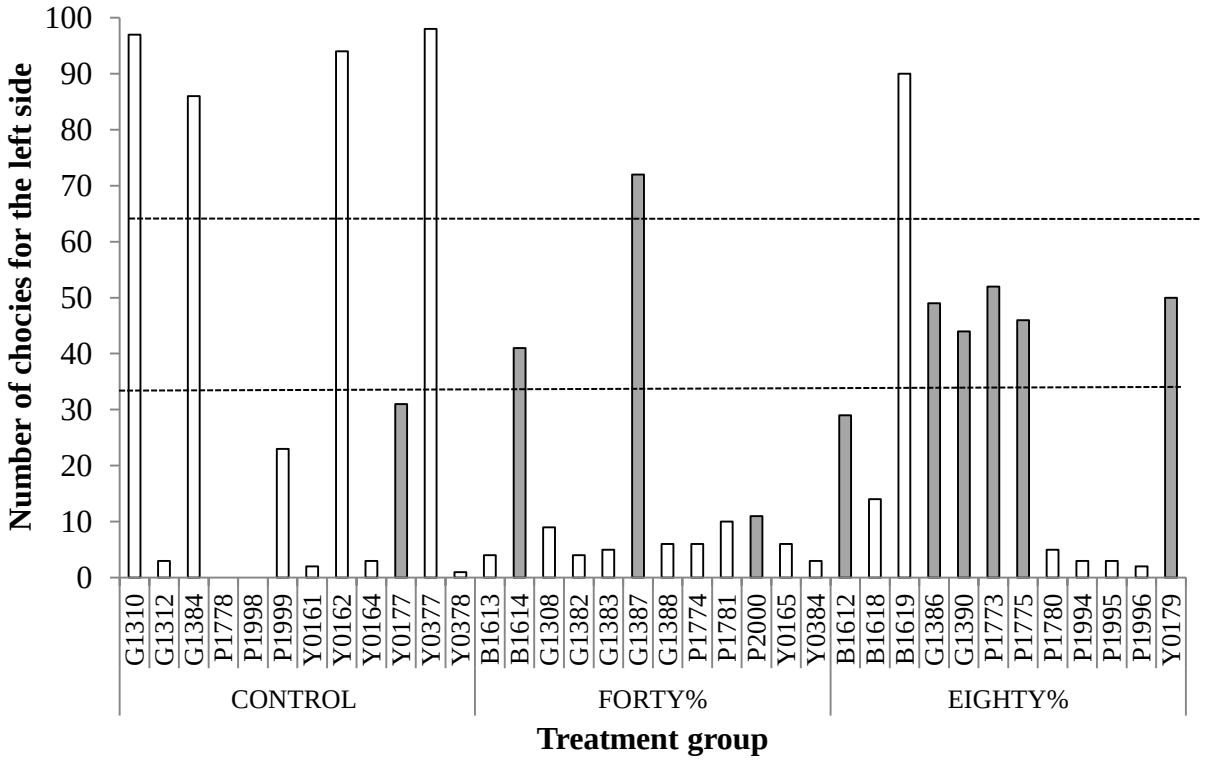
4.5.6. Side biases

Side biases were prevalent with most birds (n = 30) demonstrating a highly significant side preference (i.e. they selected their preferred side more than 66 times out of 100 trials, $P < 0.001$). Figure 5 illustrates the severity of the side biases where present with most birds picking their preferred side on more than 90% of all trials.

All of the birds that did not meet the criterion for success (n = 26) demonstrated a highly significant side bias ($P < 0.001$). Of the birds that did meet the 'success criteria', four also showed a highly significant side preference ($P < 0.001$).

Figure 5: Prevalence of side biases over the 100 trials, as indicated by the number of choices for the left side of the Y – maze, according to each individual bird in each treatment group.

The grey bars indicate the birds that met the criterion for success (picking the larger option ≥ 15 / 20 times in a single block) and the white bars indicate that they did not meet this criterion. The study was balanced such that the larger diet option could be found at the end of the left and the right arm of Y – maze an equal number of times. The dashed lines indicate the highly significant ($P < 0.001$) threshold (< 34 / 100 choices for the left side or > 66 / 100 choices for the left side). NB. Bird P1773 (Eighty% group) was euthanised after 60 trials. Her number of choices have been adjusted (original: 31 / 60) and all results are therefore expressed as a proportion.



An analysis of the two birds that met the inclusion criterion initially but then lost this preference indicated that the birds deviated from their chosen side preferentially to select the colour associated with the larger feed reward. The Forty% bird deviated from her preferred side (right side) nine times in 100 trials, in all cases when the larger feed reward was on the left side (9/9 deviations occurred when the larger food reward was associated with the left side, $P = 0.02$).

The Control bird that initially met the inclusion criteria significantly preferred the right side overall (69 choices for the right side, $P < 0.001$). Over the first five days of the free choice

tests (she met the success criterion in block one, days 1 – 4) she deviated from the right side only when the left side was associated with the larger feed reward (8 / 8 deviations, P = 0.008)

4.6. Discussion

The data indicated clearly that chronic feed restriction negatively affected the birds' ability to learn a complex feed quantity discrimination task. However, feed restriction did not totally prevent the birds from learning anything. Rather, it influenced what was learnt. The birds entered a goal box faster (irrespective of treatment) with time indicating that the all birds learnt to associate the end of the Y – maze with a positive outcome (food) and that there was an increase in associative strength with the number of trials. Increased speed seems unlikely to be due to larger birds being able to run faster (the size of the birds increased over the experiment; Figure 2). In support of this it was noted that the weight of the Controls at the end was similar to the Forty% birds at the start of the trial but the latency was shorter in the Controls at the end compared to the Forty% birds at the start. Thus, in the absence of learning the association, the default strategy of 'unsuccessful' birds appeared to be to access feed as quickly as possible.

These findings are interesting when placed in the context of other research that has investigated the effect of feed restriction on cognitive processes (e.g. Nicol & Pope, 1993; Diano *et al.*, 2006; Ferreira *et al.*, 2006; Deng *et al.*, 2009). Broadly speaking, the effects of feed restriction can be divided into two areas: firstly, those caused by nutritional stress (specific nutritional deficits having effects on the animals physiological status) and, secondly, those caused by psychological stress (the experience of hunger). The broiler breeders in our study were experiencing different degrees of feed restriction from two weeks of age. In a review of the cognitive effects of early malnutrition, Strupp and Levitsky (1995) report that early nutritional stress is associated with increased emotional reactivity, cognitive inflexibility, and attentional changes with a more narrow focus of attention that endures post-malnutrition. These suggest that malnutrition is associated with changes in neuronal development, so this is a plausible explanation for our findings. However, it is important to note that commercial broiler breeders are feed restricted to levels equivalent to our controls to optimise physical health and no other physical signs of mal – or under – nutrition have been identified. Although it remains possible that there are negative effects of feed restriction on broiler breeder cognition despite the birds being in good health, it seems more likely that treatment differences in this study resulted from hunger stress rather than direct nutritional effects on brain development.

Most studies using feed restriction as the psychological stressor or motivator do not use food – rewarded tasks when examining the effects of feed restriction on learning and memory. This is presumably because as feed restriction increases the motivation to obtain food (Conrad, 2010) the hunger-stress and motivation effects on performance would be confounded. Where food has been used as a reward, the task has been a simpler food – no food reward paradigm. In such cases, a positive association was found between the degree of feed restriction and task acquisition, because of the effect of restriction on motivation (Eisman *et al.*, 1956: deprived of food for 4h, 22h and 45½h before training; Richman *et al.*, 1970: adult rats feed restricted to maintain 75, 85 and 95% of *ad libitum* feed bodyweight). To the authors' knowledge, there is no published research looking at the effect of feed restriction on ability to learn a feed restriction – relevant complex discrimination task in which both options are food rewarded. This may be, in part, due to the confounding variable of learning ability and motivation in this type of task. However, there are relatively few studies that ask an animal to learn a discrimination task in which both options are rewarded. However, there are occasions in which this approach may be appropriate and determining the preferences of feed restricted broiler breeders for qualitative or quantitative dietary restriction is one of these. The effects of feed restriction on broiler breeder learning ability could have been quantified in a non – food rewarded paradigm. However, the results of this approach may not have translated into learning ability when faced with a task that is likely to be highly arousing to severely feed – restricted birds.

The Yerkes – Dodson Law predicts that the optimal arousal level for learning difficult tasks will be lower than for simple tasks (Yerkes & Dodson, 1908). Whilst this study was not an explicit test of the Yerkes-Dodson Law, the results partially support this interpretation: a lower level of arousal (i.e. a lower level of chronic feed restriction) was necessary for successful learning of this complex task. By comparison, in a previous study (Buckley *et al.*, 2010) more severely restricted birds (equivalent to the Controls in this study), were able to learn a simpler food / no food task.

In some studies using non – food rewarded tasks in rats, different levels of chronic feed restriction resulted in an inverted U – shape relationship with learning or memory or both (Ferreira *et al.*, 2006: Deng *et al.*, 2009). Ferreira, *et al.* (2006) compared memory and learning in rats chronically feed restricted to 70% and 40% of *ad libitum* intake with rats fed *ad libitum* throughout the study. They found that feed restriction improved learning but that the less severely feed restricted rats showed evidence of more rapid avoidance learning and showed improved memory of this learning than either the *Ad libitum* or 40% feed restricted

rats. Deng *et al.* (2009) found that mild feed restriction ((80% of *ad libitum* intake) resulted in improved long term memory, whereas it was unchanged from *ad libitum* performance in rats feed – restricted to 40% and 20% of *ad libitum* intake. Different levels of injected ghrelin (an appetite-stimulating hormone) produced similar effects when mice were tested on T – maze tasks (Diano *et al.,* 2006). This indicates that cognitive processes are sensitive to the degree of feed restriction. Further, synapsin – deficient animals (synapsin proteins are associated with learning and memory) have been shown to be memory – impaired (for example see: Michels *et al.,* 2005; Porton *et al.,* 2010). Deng *et al.* (2009) found that synapsin production was up – regulated with relatively mild feed restriction (80% of *ad libitum* intake), similar to *ad libitum* levels at 60% restriction but down – regulated with increasingly severe feed restriction (40% & 20% of *ad libitum* intake). However, in the present study there was no evidence of an inverted – U – shaped pattern to task – learning ease. Instead, the relationship was linear: the less hungry the bird, the greater the likelihood that it would learn the task. This was interesting because although the 80% group were the least 'hunger-stressed' they would also have been the group least motivated by a food reward. It is possible that this inverted – U – shaped curve could have been observed if *ad libitum* fed birds had been used in the choice test but this seems unlikely. The 'successful' birds in the 80% treatment group frequently did not eat the entire portion of feed they had access to which suggests that, although motivated to access food rewards, these birds were not as hungry as the other treatments. Low levels of arousal may partially explain why half of the 80% group failed to learn the task. However, overall, the least hungry birds found the task easiest to learn.

In our study we believe that the birds on different treatments differentially experienced hunger – stress. Although we did not formally measure either physiological or behavioural indicators of stress there is sufficient scientific evidence to support this interpretation (e.g. Hocking *et al.,* 1993; Hocking *et al.,* 1996; de Jong *et al.,* 2002; de Jong *et al.,* 2003). In agreement with these reports we observed spot – pecking, stereotypic behaviour (pacing) and excessive drinker manipulation in our control birds indicating that the birds were frustrated by being feed – restricted. It is considered that, in addition to the chronic stress of feed restriction, the birds' also experienced acute arousal during training and testing (due to the association between the apparatus and food). Acute stress or arousal may enhance learning where there is convergence in time between the stressor and the learning task and where learning the task removes the stressor (Joels *et al.,* 2006; but see Schwabe & Wolf, 2010). However, chronic stress is a potent negative barrier to effective learning and memory (Joels *et al.,* 2006; Conrad, 2010). Severe levels of chronic feed restriction (50% of *ad libitum*

intake) are associated with poor cognition in juvenile rats (Young & Kirkland, 2007). This level of feed restriction roughly approximates to our 40% group during training / testing. The Control group were considerably more feed restricted (circa 25 – 33% of the intake of *ad libitum* fed birds of the same age).

In our study, the task *was* food rewarded and therefore relevant to reducing the stress of feed restriction. This aspect should have increased the likelihood of the birds learning the task. The potential relative gains (proportional extra daily feed allowance) were higher for the Control group. Therefore, these birds had a much greater incentive to learn. However, this was not realised in terms of performance in the Y – maze.

Schwabe, *et al.* (2010) point out that stress affects both the quantity and quality of the learning experience. Stress affects the quantity of information processed in various ways that lead to both increased and reduced performance in cognitive tests dependent upon what is measured (Mendl, 1999). The quantity of information processed by the birds may have been negatively affected by chronic feed restriction in several ways.

Firstly, the birds may have experienced attentional narrowing. Attentional narrowing can be defined as focusing of attention on the central features of a task or event whilst ignoring more peripheral or less salient features of the same task or event (see Mendl, 1999). Severe chronic feed restriction in growing rats leads to a failure of the rats to attend to environmental stimuli (reduction in perceptual learning) that were apparently unconnected to immediate biological needs (Rogers *et al.*, 1986). Stress also decreases attendance to 'redundant' cues (cues that are introduced to a training situation which provide no additional information regarding the correct response (Levitsky (1979), reported by Rogers *et al.* (1986). These studies indicate that 'attentional narrowing' can occur under conditions of stress. Easterbrook (1959) highlights that when peripheral cues are important for task performance, ignoring these cues leads to performance error. However, it is also possible that, in our study, the birds that failed the task did so because they attended to too many cues and, therefore, failed to sufficiently attend to the cues that were relevant to learning the task. For example, they may have focussed on the Perspex barrier (whilst in the start box) or the terminal pens and / or their contents (at any point during each trial).

Secondly, Mendl (1999) suggests that stress / arousal may increase errors by speeding up decision – making such that the animal does not attend to all the relevant information before making a decision. Although birds were given an enforced period of observation before making a decision (30 seconds in the start box) the birds may have attended more to the Perspex barrier between them and food and only attended to runway stimuli once released. In

our study, the hungriest birds ran faster, probably reflecting their greater motivation. Therefore, these would have had less time to assimilate the colour – information.

Finally, holding birds in the start box for 30 seconds before release may have further increased arousal resulting in a concurrent reduction in learning. Van Rooijen & Metz (1987) found that feed restricted pigs working for food in a food – no food T-maze task failed the task when held in the start box for 5 minutes. Reducing the time to 30 seconds significantly improved performance (although this was confounded with the additional number of trials). Thus, holding the birds for less time might have improved performance. However, we consider this unlikely as the birds (especially controls) were highly aroused generally and focussed on trying to escape both the home pens (prior to testing) and the start box. Therefore, we think it is more likely that they simply did not process the relevant information prior to release from the start box.

It is likely that the poor performance also reflected changes to the quality of learning. The default strategy of all birds that failed to learn the task was to show a positional bias irrespective of treatment. Chickens have been demonstrated to show low levels of spontaneous alternation (Hughes, 1989; Haskell *et al.,* 1998). Increased perseverance in this study was reinforced by the provision of a food reward. With hindsight, this was a study weakness that could be addressed in any future work utilising a Y-maze, through the use of forced trials and occasional 'probe' choices in which the animal could choose which arm to enter. Nonetheless, positional biases were more common among the hungrier treatments.

The stress of feed restriction may have reinforced the tendency to persevere as a function of habit. Hunger stress has been associated with positional biases in both pigs (Van Rooijen & Metz, 1987) and starlings (Talling *et al.,* 2002). Shocking mice upon entry to one of the goal boxes in a T – maze reduced stimulus – response learning (avoidance of shocks) and increased habitual responding that resulted in increased exposure to electric shocks (Mitchell *et al.,* 1985). In food devaluation studies, stressed humans show a habitual, rather than a goal – directed, strategy (Schwabe & Wolf, 2009). In our study, one Control bird and one Forty% bird initially showed a significant preference for the larger reward in the first trial block after the forced trials but then developed strong side biases. The pattern of deviations from the preferred side suggests this initial preference was genuine and not due to chance. As the trials progressed it is expected that the level of feed restriction became increasingly severe. Stress – induced habitual learning may have resulted in the much poorer performance in subsequent trial blocks, including reluctance to change behaviour even when quantity contrast was increased.

Despite the fact that feed restriction did affect the birds' ability to learn the task in this experiment, it is also clear that it was difficult to learn even for birds in the least feed restricted group. It is not clear why. However, several factors may have affected this: the tendency of chickens to show low spontaneous alternation (Hughes, 1989; Haskell *et al.*, 1998) combined with the free choice methodology in which side biases were reinforced by food rewards may have stimulated the likelihood of side biases. Alternatively insufficient trials, inter – trial length (but see Sarason *et al.*, 1956; D'Amato, 1960) or absolute or relative food quantities (the smaller the contrast the more difficult discrimination will be and the longer the task takes to learn. See: Hill & Spear, 1963; Clayton, 1964) may have affected performance. However, it is worth noting that most of the 'successful' birds had learnt the task within 60 free choice trials. Alternatively, a species specific difficulty associating relatively distal colour cues with food quantities rather than food location *per se* could have affected task performance. However, Phillips & Strogan (2007) found layer hens could associate feed qualities with colours with far fewer trials. This suggests that the task was potentially learnable. Combined with our previous work (Buckley *et al.*, 2010), the current study suggests that the free-choice Y – maze method used here would not be particularly useful in evaluating broiler breeder preferences for qualitative or quantitative dietary restriction.

4.7. Conclusions and animal welfare implications

Chronic feed restriction is a potent stressor that negatively affected broiler breeder performance in this complex task, even though the task was relevant to removal of the stressor, a factor known to improve task success. Therefore, most of the birds did not express a preference for a larger food reward in this choice test. This study further demonstrates the negative effects that commercial feed restriction has on broiler breeders, in this case by reducing the broiler breeders' performance in a food – quantity discrimination task. Although the mechanism leading to reduced performance is unclear, in the wider context, this may provide additional support for the concerns (Mendl, 1999) that, firstly, stressors may render choice tests for identifying 'wants' problematic and, secondly, stressors may negatively affect an animal's ability to function in its environment by reducing its ability to assimilate information favourable to its welfare.

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5. The use of conditioned place preference to determine broiler preferences for quantitative or qualitative dietary restriction

5.1. Preamble

This experimental chapter has been accepted for publication in the peer – reviewed journal British Poultry Science. The relevant reference is:

Buckley, L. A., Sandilands, V., Hocking, P. M, Tolkamp, B. J. and D'Eath, R. B. (In press) The use of conditioned place preference to determine broiler preferences for quantitative or qualitative dietary restriction, *British Poultry Science*

Excerpts of this chapter have also been presented at two conferences. The relevant references are as follows:

Buckley, L. A., Sandilands, V. S., Hocking, P. M., Tolkamp, B. J. and D'Eath, R. (2011) Can conditioned Place Preference be used to determine hungry broiler preferences for qualitative or quantitative feed restriction. *World Poultry Science Association (UK branch) Annual Meeting*, 4th – 5th April, Nottingham, UK [oral presentation]

Buckley, L. A., Sandilands, V., Hocking, P. M., Tolkamp, B. J. and D'Eath, R. (2011) Conditioned place preference: a tool to determine hungry broiler diet preferences. *45th Congress of the International Society of Applied Ethology*, 31st July – 4th August, Indianapolis, USA [poster presentation]

5.2. Abstract

Calcium propionate (CAP) may improve the welfare of feed restricted broiler breeders by improving their satiety when included within the feed ration. However, the evidence for this is mixed.

This study used a closed economy conditioned place preference (CPP) task and aimed to identify whether broilers (as a model for broiler breeders) preferred an environment associated with quantitative food restriction (QFR) or an environment associated with a diet quality – adjusted by the inclusion of CAP. Birds taught to associate different environments with QFR and *ad libitum* (AL) access to feed were used to validate the methodology.

The two treatment groups were 1) QFR/AL (n = 12) in which birds alternated every two days between QFR and *ad libitum* access to food, and 2) QFR/CAP (n = 12) in which birds alternated every two days between QFR and QFR + calcium propionate (increased from 3 – 9% over the study period). Birds were taught to associate one diet option with vertical stripes and the other with horizontal black and white stripes. Each bird was tested twice for a CPP (once per diet).

QFR/AL birds showed a significant preference for the pen associated with *ad libitum* access to feed, but only when tested hungry (i.e. fed QFR on day of testing). QFR/CAP birds did not show a preference under either hunger state.

Reasons for the failure of QFR/CAP birds to show a preference are unclear but could include a lack of preference or failure to learn the task. The existence of state – dependent effects indicates that care is needed in the design of future CPP studies and that the effect of calcium propionate and level of hunger on ability to learn a CPP needs further investigation.

5.3. Introduction

Broiler breeders of fast growing strains of broilers (the most common commercial lines) are feed restricted to ensure that the bird has a healthy rate of growth and maximal rates of fertility (Savory *et al.,* 1993). This feed restriction is both severe, with birds fed as little as 25 – 45% of *ad libitum* intake (dependent on whether comparisons are made between age – or bodyweight – matched birds; Savory *et al.,* 1993), and chronic, with birds being feed restricted to various degrees from about 1 week of age until the end of their productive life (circa 18 months; based on manufacturer performance objectives, Aviagen, 2007). The available behavioural and physiological evidence indicates that these birds experience chronic hunger (Savory *et al.,* 1993; Savory and Maros, 1993; Hocking *et al.,* 1993, 1996, 2004; De Jong *et al.,* 2003). With around 6.3 million broiler breeders being reared in 2010 alone in the UK (DEFRA, 2011) feed restriction is a major welfare issue within the meat bird industry.

Quality – adjusted diets that take longer to consume potentially improve feed restricted broiler breeder welfare by increasing satiety and allowing more naturalistic foraging behaviour to occur (for a review see D'Eath *et al.,* 2009). However, there is a need for further research to quantify this perceived benefit by using methods that identify the relative affective state of the broiler when fed either a quantitatively or qualitatively restricted diet. One potential dietary adjustment that might improve levels of satiety is the addition of propionate – containing compounds such as calcium propionate (CAP) (Arora *et al.*, 2011). Propionate has been linked to increased feelings of satiety in humans (Arora *et al.,* 2011). Experimentally, the addition of CAP to broiler feed has been shown to reduce feed intake by up to 25% when fed to immature (4 – 8 week old) broiler breeders at a 3% inclusion rate (Kapkowska *et al.,* 2005) although this declines to about an 8% reduction in feed intake by 18 weeks of age. Sandilands *et al.* (2006) achieved a larger voluntary reduction in feed intake by increasing the inclusion rate from 5 – 10% over the rearing period, although the bodyweight of these birds was still significantly greater than birds reared using quantitative feed restriction to commercial levels at 6 and 12, but not 18 weeks of age.

However, the mechanism by which propionate achieves this reduction of food intake is unclear. It is thought to act by delaying gastric emptying and / or by various post – absorption effects on metabolism (Arora, *et al.,* 2011; but see Darzi, *et al.,* 2011). One plausible hypothesis is that this results in a sensation of satiety (a positive affective state)

which birds find rewarding. Alternatively though, birds may find eating or utilising food containing CAP unpleasant in some way. Darzi, *et al.* (2008) found that when propionate was administered orally in a palatable form to humans there was no suppression of appetite. Metabolic acidosis might be induced at high inclusion rates, but at the low levels typically used this is not a problem (Pinchasov and Elmaliah, 1994). Oral lesions have been observed in some studies (Tolkamp *et al.*, 2005; Bolton and Dewar, 1964) suggesting oral discomfort as a mechanism but this is not always observed (Buckley *et al.*, unpublished data). The fineness of mash diets, which is exacerbated by the inclusion of CAP, has also been implicated in the aetiology of oral lesions (Gentle, 1986; Tolkamp *et al.,* 2005). Studies which bypass the gastrointestinal tract by injection of propionic acid (the active ingredient) also achieve appetite suppression (Pinchasov and Jensen, 1989). Previous work by Buckley *et al.* (2011a) found that diets containing 3% calcium propionate were less preferred compared with an otherwise identical basal diet by broilers in a two – pan simultaneous choice test. However, this preference may be sensory – led and not reflective of the affective state of the bird post ingestion (i.e. does it increase satiety relative to quantitative dietary restriction?). To summarise, the effect of CAP on feed intake might be a consequence of increased satiety (a positive affective state) or alternatively result from an aversion that results in the animal delaying consumption despite being hungry (a negative affective state). This issue needs to be addressed before a claim that CAP improves the welfare of feed restricted broiler breeders can be made.

The current study uses Conditioned Place Preference (CPP) to investigate whether broiler chickens find food containing CAP aversive or rewarding. CPP methodologies are based on the principles of Pavlovian conditioning: an animal can be conditioned to prefer a previous neutral, or un – preferred, environment by pairing it with the presence of something that the animal finds rewarding (Tzschentke, 1998). It is an approach widely used within the pharmaceutical industry (reviewed by Tzschentke, 1998; Bardo and Bevins, 2000) to investigate the effects of various pharmaceutical agents on the affective state of the animal. The animal is injected with the compound and then immediately placed within the distinctive environment to be conditioned. If the drug results in a positive affective state then the animal will prefer this environment over one it is placed into after an injection of saline (which has no effect on affective state). However, CPP has also been demonstrated to occur as a consequence of natural reinforcements including food (e.g. Spyraki *et al.,* 1982; Papp, 1988, 1989; Imaizuma, *et al.,* 2000, 2001; Dickson *et al.,* 2010; Matsumura *et al.,* 2010). Whilst most studies are in rodents, CPP have been demonstrated in avian species including quail

(Mace *et al.,* 1997; Akins *et al.,* 2004) and chickens (Bronson *et al.,* 1996; Hughes *et al.,* 1995). Recent work by Dixon *et al.* (2011) suggested feed restricted broiler breeders can learn a CPP using aversive stimuli that they are exposed to for several days in a closed economy environment. Y – or T – maze type choice tests in which feed restricted broiler breeders have to choose between different quantities or qualities of food that were associated with distinctive arms of the maze proved unsuccessful in previous research (Buckley *et al.,* 2011a, 2011b). Thus, it is appropriate to investigate methods in which the animal is in contact with the 'to – be – conditioned' stimuli for longer (e.g. at least eight days, Dixon *et al.,* 2011) compared to the time spent in a Y – maze arm (which may be as little as 2 seconds in commercial feed restricted broilers before they make a choice and exit the maze, Buckley *et al.,* 2010b). Also potentially beneficial is that in CPP testing the animal is tested in extinction (i.e. with no food present that is likely to elicit impulsive behaviour). These methods may be more successful at determining broiler breeder preferences for quantitative or qualitative dietary restriction. Further, training and testing the animal in a closed economy conditioned place preference apparatus may allow preferences based on the whole experience of each diet 'system' to be identified. This is because the animal will be in contact with the 'to – be – conditioned' stimulus post – consumption of the diet. Thus, the animal should express a preference based on its overall affective state (primarily how hungry or satiated the bird felt post-diet consumption) on days during which it experiences quantitative (or qualitative) dietary restriction.

The current study hypothesised that feed-restricted broilers would show a preference when given a choice between environments associated with quantitative dietary restriction (QFR) or qualitative dietary restriction (diet containing CAP) in a closed economy CPP task. It was expected that the direction of this effect would indicate whether the broilers found a diet quality-adjusted with the addition of CAP more or less aversive than commercial levels of quantitative dietary restriction. To validate the study methodology, a second group of birds were given a choice between environments associated with *ad libitum* (AL) access to food or QFR. It was expected that the birds would show a preference for the *ad libitum* feed access environment. Novelty (preference for pen not housed in on the day of testing) was identified as a problem in previous work by Dixon *et al.* (pers. comm). Therefore, here both groups of birds were tested twice (once on a day when fed QFR and known to be in a state of hunger and once on a day when fed the alternative diet option and in a state of satiety (*ad libitum* fed birds) or an unknown state (CAP-fed birds)). This enabled the identification of pen – novelty related effects. This also enabled the identification of any state – dependent preferences. It

was predicted that state dependent effects would not be present as it was expected that birds would always prefer the environment that they associated with feeling satiated over one that they associated with feeling hungry.

5.4. Methodology

5.4.1. Subjects

Twenty – four female Ross 308 broiler chicks were used and recruited to the study as 28 day old birds. Broilers were used here as a more readily available model for parent stock. Prior to this study the birds had been group reared on a 14:10 h light: dark schedule (day 1 – 28) and spot-brooded (day 1: 31˚C, reduced gradually to 21˚C on day 21 and maintained at this temperature thereafter). The birds were fed a commercial starter chick crumb (Farmgate, BOCM Pauls Ltd., Ipswich, Suffolk, UK) *ad libitum* from 1 – 14 days and, thereafter, feed restricted in line with the recommended daily feed requirements for broiler breeders (Aviagen, 2007). The mean (standard deviation) bodyweight of the birds on admittance to the study was 528.8g (± 32.3g) which was 20% heavier than the target bodyweight for broiler breeders at 28 days (440g). They had no previous experimental history.

A study timeline is provided to provide a brief overview of the experimental design and use of the experimental subjects (Table one).

Table 1: Study timeline showing key information relating to the experimental design

<table>
<tr><th>Day</th><th>0 - 27</th><th>28</th><th>28 – 43</th><th>44 - 67</th><th>67</th></tr>
<tr><td>Phase</td><td>Pre-study</td><td rowspan="8">Start CPP experiment

Allocate birds to treatment groups</td><td>Pre-CPP</td><td>CPP</td><td rowspan="8">End CPP experiment

Birds maintained on the same every other day alternating diet option schedule

Day 93: Euthanasia and post-mortem of all birds</td></tr>
<tr><td>Housing</td><td>Group - housed</td><td colspan="2">Day: individually housed
Night: group housed</td></tr>
<tr><td>Treatment groups</td><td>N/a</td><td colspan="2">1. QFR/CAP (n = 12)
2. QFR/AL (n = 12)</td></tr>
<tr><td>Diet options</td><td>All: commercial starter pellet</td><td colspan="2">1. Mash grower diet ±calcium propionate for QFR/CAP birds
2. Mash grower diet - feed restriction or ad libitum access (QFR/AL birds)</td></tr>
<tr><td>Diet protocol</td><td>Fed AM, feed restricted from 15 days</td><td colspan="2">All birds:
Alternate every second days between QFR and alternative diet option i.e. AA, BB, AA, BB, AA, …</td></tr>
<tr><td>Pen design</td><td>N/a</td><td>Plain walls</td><td>Vertical and horizontal black and white striped walls</td></tr>
<tr><td>Pen protocol</td><td>N/a</td><td>Alternate every other day between right and left pen i.e. A,B,A,B,A, …</td><td>Alternate every second day between the right and left pen (in line with diet option switches) i.e. AA,BB,AA,BB, …</td></tr>
<tr><td>Test protocol</td><td>N/a</td><td>All birds:
4 side bias tests: once per bird on days 37, 38, 39 & 40</td><td>All birds:
2 CPP tests: Once per bird on days 55 & 67</td></tr>
<tr><td>Diet option fed on day of test</td><td>N/a</td><td>All birds:
2 tests per bird on days when fed QFR; 2 tests each per bird on days when fed the other diet option</td><td>All birds:
1 test per bird on a day when fed QFR; and 1 test per bird on a day when fed the other diet option</td></tr>
</table>

5.4.2. Treatment groups

Birds were blocked according to weight, and then randomly allocated to one of two treatment groups on day 28. The treatment groups were: 1) QFR versus qualitative feed restriction (in which the food had calcium propionate added; QFR/CAP, n = 12), and 2) QFR versus *Ad libitum* feed (QFR/AL, n = 12). Each bird thus experienced two different diets, with which two diets offered dependent on treatment group. There were two phases to the

experiment: 1) pre-CPP stage, and 2) CPP training and testing. The diet regime remained the same across both phases.

5.4.3. Diet and feeding regime

Irrespective of treatment, all birds were fed at 09:00h. Feed remaining at 17:00h was removed and weighed. Daily feed intake was recorded for each bird. All birds alternated every two days (from day 28 – 67 (end of study)) between the two diet options assigned to their treatment group. Within each treatment group, half the birds received QFR on the first two days followed by the alternative diet option (CAP or AL) on the following two days, and alternated thereafter (n = 6 per treatment). The other half of the birds received these diet options in the reverse order (n = 6 per treatment).

The basis of all the diets was a custom – made grower mash (Target Feeds, Whitchurch, Shropshire, UK) suitable for broiler breeders from 28 days of age. The diet was formulated to contain 165g/kg crude protein and 12.1MJ ME/kg feed. Birds in the treatment group QFR/AL received only this diet. They alternated every second day between QFR and *ad libitum* access to this diet between 09:00h – 17:00h. Birds in the treatment group QFR/CAP alternated between QFR and a diet that was qualitatively restricted by the inclusion of calcium propionate (CAP). The CAP option was the same quantity of diet as received under QFR *plus* the addition of calcium propionate (Propimpex® CA powder, Impextraco, Germany) mixed into the ration. The quantity of calcium propionate was increased over the duration of the study, from 30g – 90g/kg total feed (3 - 9%). The inclusion rate started at 3% based on previous work by the authors (Buckley *et al.,* 2011a) which indicated that this ration would be consumable within 8h. This was then increased to maximise time taken to consume ration whilst at the same time aiming to ensure that all birds fed that ration on that day had consumed ≥ 95% of the total ration by 17:00h. Thus, the calcium propionate level was increased to 4% on day 36 (or day 38), 5% on day 41 (43), 6% on day 45 (47), 7% on day 49 (51), 8% on day 57 (59), 9% on day 60 (62) and remained at 9% until the end of the study.

The levels of CAP were increased based on previous work by the authors (unpublished observations) and Sandilands *et al.* (2005) who found that it was necessary to increase the levels of calcium propionate included in the feed over time. This was presumably necessary as birds either adjusted to its properties or increased in relative severity of feed restriction.

Birds were observed hourly between 09:00 – 17:00h and the hour by which the full ration was consumed was noted. At the end of the day any ration remaining was weighed: if ≥ 95% of the ration had been consumed the bird was considered to have consumed the full ration

within 8h. The QFR ration was always fully consumed by 8h; however, the CAP ration was not (see figure four). During the pre – CPP phase (days 28 – 43), where the ration failed to be fully consumed by 17:00h it was left in the birds' pen overnight to allow additional time to consume the ration. On these days all birds remained individually housed overnight. During the CPP phase (days 44 – 67), surplus food was discarded at 17:00h for birds failing to meet the criterion. This applied to one bird on day 45 and three birds on day 51 with the mean daily quantity consumed (% of total ration) for these birds on these days was: 47.8g (90%) on day 45 and 48.6g (90%) on day 51.

5.4.4. Housing and husbandry

For the duration of the study, the birds were individually housed during the light hours in pens containing wood shavings, a perch and a drinker allowing *ad libitum* access to water. Birds were pair – housed overnight (with all exceptions outlined in the diet and feeding regime section).

The standard enclosure was a 0.95m (width) × 1.05m (length) × 0.8m (high) solid – sided metal pen. These pens were split down the middle with a wood divider creating two identical smaller pens measuring 0.475m × 1.05m. Each divider had a removable solid door (0.4m high × 0.25m long) set into the front bottom corner of the divider. The removable solid door was replaced with a removable mesh door for the first 10 days to reduce the initial stress of social isolation in the birds. The front of the pen comprised two sections. The top 0.4m was a full – length mesh door that could be opened to allow easy access to the pen. The bottom 0.40m was a full length solid wooden divider. Set midway along this divider was a hatchway (0.25m × 0.25m) with a guillotine door. This hatchway lined up with the central pen divider such that the divider bisected the guillotine door.

There were 12 of these divided pens in total. One bird was housed on each side of the divider (i.e. in one of the 24 smaller pens). The feed bowls were placed at the front of the pen attached to a mesh grid. The distance between the feed bowls and the floor was adjusted as periodically as the birds grew to ensure ease of access but minimise spillage of feed.

The birds' pens were within a room that was maintained at 21˚C throughout the study. The photoperiod was gradually reduced from 14h / day (day 28) to 9h / day (day 33) to ensure the birds experienced a similar light period to commercially reared broiler breeder birds who are maintained on 8h light (it was slightly longer in our study to account for end of day experimental procedures (e.g. feed removal, etc). Thereafter, birds were given 9 hours light / day (09:00h – 18:00h) with the exception of the 6 days on which pre – existing side bias

testing and CPP testing occurred (days 37, 38, 39, 40, 55 and 67). On these days the number of hours of lighting was extended until 21:00h to allow the end of day data collection to occur.

5.4.5. Pre-CPP phase

The purpose of the pre – CPP phase was to allow the birds to habituate to the test conditions (solitary housing), to adjust to the diet options on offer (to prevent dietary neophobia or initial sensory – led preferences affecting CPP formation) and to enable the identification of any birds with side – biases to allow any biases to be controlled for.

5.4.5.1. Housing protocol during the pre – CPP phase

The pre-CPP phase took place between days 28 – 43. Birds were housed individually in the divided pens between 09:00h – 17:00h. At 17:00 the door in the central divider was removed allowing the birds housed on each side of the divider to move freely between the two pens. The two birds were allowed to interact until 18:00h before being returned to their pen (if any birds had failed to fully consume the feed ration) or 09:00h (if all birds had fully consumed the feed ration). All birds were housed individually overnight if any had not fully consumed the ration in order to maintain consistency between birds. This social interaction was instigated on ethical and welfare grounds and no data was collected during this interaction. In practice, there were nine days during the pre – CPP phase in which it was necessary to house the birds individually overnight. Birds alternated daily between the two pens they were housed in to habituate them to regular changes of environment whilst preventing the association of the diet options with specific pens during the pre – CPP phase, since diets alternated every two days throughout.

5.4.5.2. Side bias testing during the pre – CPP phase

Each bird was tested 4 times for the presence of a pre – existing side bias. Side bias testing took place between 17:15h – 20:15h on days 37, 38, 39 and 40. Testing was balanced within bird with half the tests taking place on days when the bird had been fed QFR and half the tests taking place on days when the bird had been fed the alternative diet option (CAP or AL). Half the tests took place on the 1st day post switch to the QFR (or alternative diet option) and the remaining half took place one 2nd day (i.e. the day before switching to the other diet option).

The side – bias testing procedure was as follows: each bird was removed from its pen 15 – 20 minutes before it was tested for a side bias and placed in a holding pen. The bird

occupying the adjacent pen was removed and placed in another holding pen just before testing of the first bird commenced. Both pens (each side of the divider) were cleaned out and fresh wood shavings added. The feed bowl and associated attachments were removed. The door in the wooden divider was removed. The bird was then placed in a box (0.25m (w) x 0.30m (l) x 0.35m (h)) that was lined up with the guillotine door. After 30 seconds the guillotine door was raised and the bird was allowed to enter either pen and allowed to move freely between both pens for 20 minutes. The bird was then removed, returned to the holding pen and the other bird tested. Once both birds occupying adjacent pens had been tested both birds were returned to the pens. First pen entered was recorded. Each bird was observed continuously and each time the bird changed pens this was recorded in seconds using a stopwatch. A bird was considered to have changed pens when both feet had entered the neighbouring pen.

The criterion for a bird being considered to have a pre-existing side bias was more than 60% spent on a particular side out of the total amount of time the bird was observed for (80 minutes). This 60% threshold was based on the work of Dixon *et al.* (pers. comm.). Within the QFR/AL group 4 birds had a right sided bias and 5 birds had a left sided bias. Within the QFR/CAP group 6 birds had a right-sided bias and no birds had a left-sided bias.

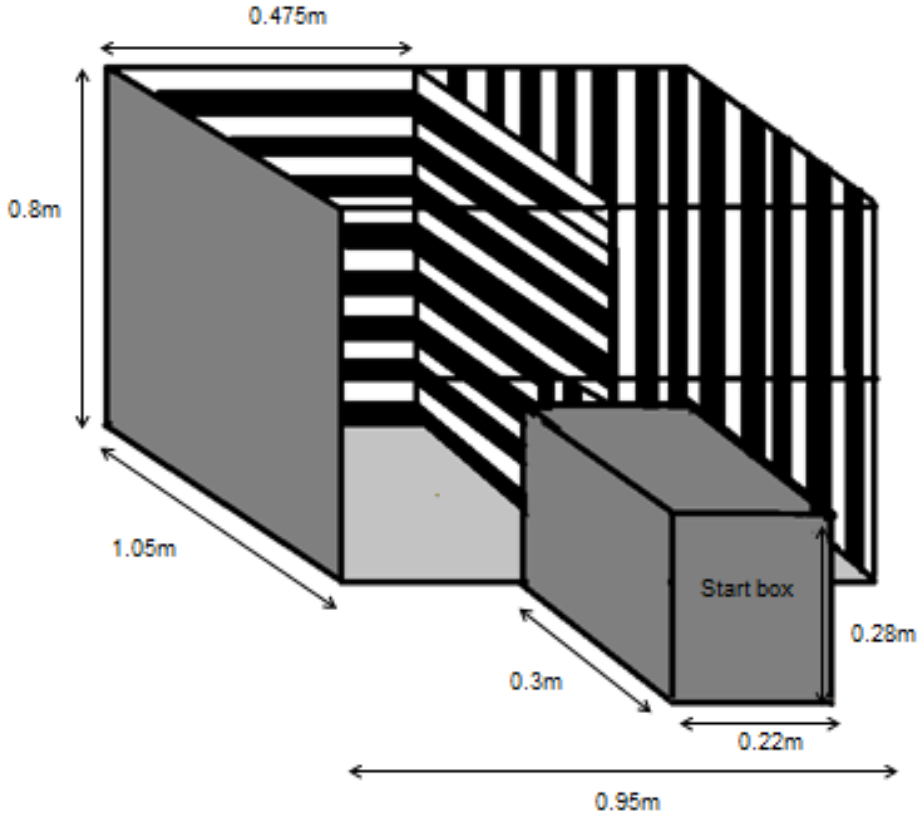
5.4.6. CPP phase

This phase comprised CPP training and both tests for the existence of a CPP.

5.4.6.1. CPP apparatus

The CPP apparatus was the same divided pens as used for the pre – CPP phase as described above but covered from floor level to a height of 0.7m on the side and back walls of the pen with sheets of varnished and laminated paper. Each adjacent pen (i.e. separated from each other by the divider) had paper sheets with one of two patterns. In one pen the pattern was vertical black and white stripes (33mm wide stripes), while in the other pen it was horizontal black and white stripes (16mm wide stripes; Figure 1). This was balanced such that half the 'vertical – striped' pens were on the right side of the divider and the 'horizontal – striped' pens on the left side and vice versa. Both pens were designed to ensure an equal coverage of black and white to control for brightness.

Figure 1: Diagram of CPP apparatus (not drawn to scale). The front of the pen (demarked by the two horizontal stand-alone lines) has been cut away to allow the reader to better visual the pen set – up during CPP testing.



5.4.6.2. CPP training and housing protocol

On day 44, CPP training began and lasted until day 67 (end of the study). Each bird lived in one of the distinctive pens on days when it received QFR and the other distinctive pen on days when it received AL (QFR/AL treatment group) or CAP (QFR/CAP treatment group). The aim was to allow the birds to associate the different pens with the state of hunger that they experienced within them. Within treatment, half the birds (n = 6) experienced QFR in the vertically striped pens (for three birds this was the right sided pen and for three birds this was the left-sided pen) and half (n = 6) experienced QFR on the horizontally striped pens (also balanced for pen side). To control for pre – existing side biases birds that demonstrated a pre – existing side bias were approximately equally distributed such that, within each treatment group, half the birds received QFR on their preferred side and half received QFR on their least preferred side.

Birds lived in the CPP apparatus continuously. Between 09:00h – 17:00h birds were housed individually. Overnight, they were pair – housed with another bird. The conspecific they were

housed with remained the same throughout this phase. Birds were paired according to treatment group and pen pattern experienced that day (i.e. a bird that had been housed in a vertical striped pen was housed overnight with another bird that had experienced vertical stripes that day and both birds were from the same treatment group). On days when birds were switched between environments they were switched at 09:00h before being fed.

5.4.6.3. CPP testing

Each bird was tested for a CPP after 12 days of training (6 days per diet: environment pairing; tested on day 55) and 24 days of training (12 days per diet: environment pairing; tested on day 67). The CPP testing protocol was identical to the protocol for side bias determination. See figure one for a visual representation of the CPP test apparatus.

5.4.7. Statistical Analysis

All statistical analyses were undertaken using Genstat (13th Edition, VSN International, Ltd., Hemel Hempstead, UK). The Shapiro-Wilks test was used to evaluate the distribution of proportion data which was normal. Therefore, untransformed data and a repeated measures REML were used to investigate any differences between treatments, effect of state at the time of testing and interactions between these. Number of pen changes was normalised using the log-e transformation prior to REML analysis. For all analyses, subject was the bird ID. The relevant time point was test number and these were equally spaced and identical between subjects. The fixed effects were: treatment, diet option fed on day of testing, pattern associated with QFR and interactions between these. The variables of interest tested were: proportion of time spent in the non – QFR pen; proportion of time spent in the 'novel' pen; proportion of time spent in the right pen. Differences from 0.5 were evaluated using the One-sample T – test. Where confidence intervals are reported these are at the 95% significance level.

First pen entered data was tested using a GLMM with a logit-transformed binomial distribution. The variables of interest included: which pen was entered (associated with QFR or alternative diet; right or left sided pen; novel pen or pen bird spent the day of testing in). The fixed effects examined included: treatment, state at time of testing; pattern associated with QFR and all interactions between these. The random effect was bird ID. Differences from 0.5 were calculated by chi-square (1 d.f.) using a test statistic generated by the following formula: χ^2 = (predicted mean / S.E. of the predicted mean)^2. Predicted means were generated by GLMM.

5.4.8. Ethical considerations

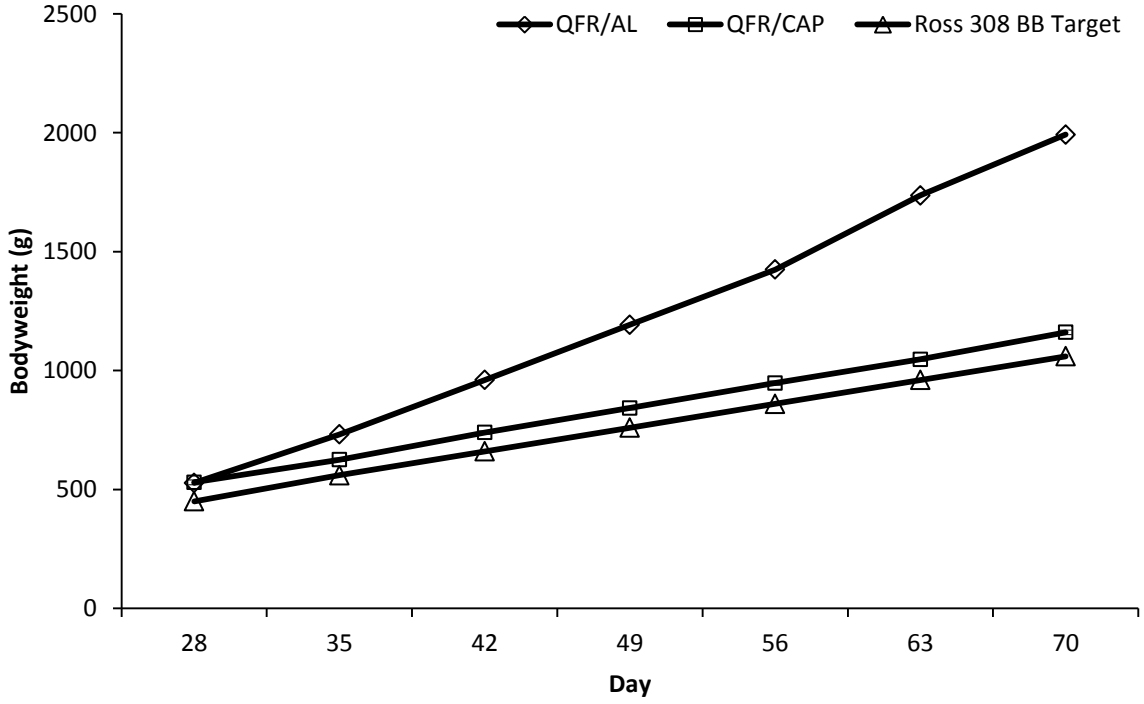
This study was carried out under Home Office license and was approved by both the Scottish Agricultural College's and Roslin Institute's Animal Ethics Committees. Pen sizes exceeded the minimum recommendation for individually housed poultry and shavings and a perch were provided to facilitate natural behaviour. Due to the study design, it was considered necessary to house birds individually during the day; however, birds were pair – housed overnight (17:00 – 09:00h) for the majority of the study to allow for some social interaction as it is recognised that chickens are a social species. Feed restriction was no more severe than under commercial conditions (and, in the case of the QFR/AL birds far less severe). All birds remained healthy during the study. At 93 days of age birds were sent for a post – mortem to assess any potential gastrointestinal tract pathology as a possible consequence of calcium propionate ingestion. No treatment – related pathology was identified.

5.5. Results

5.5.1. Growth curves and feed intake

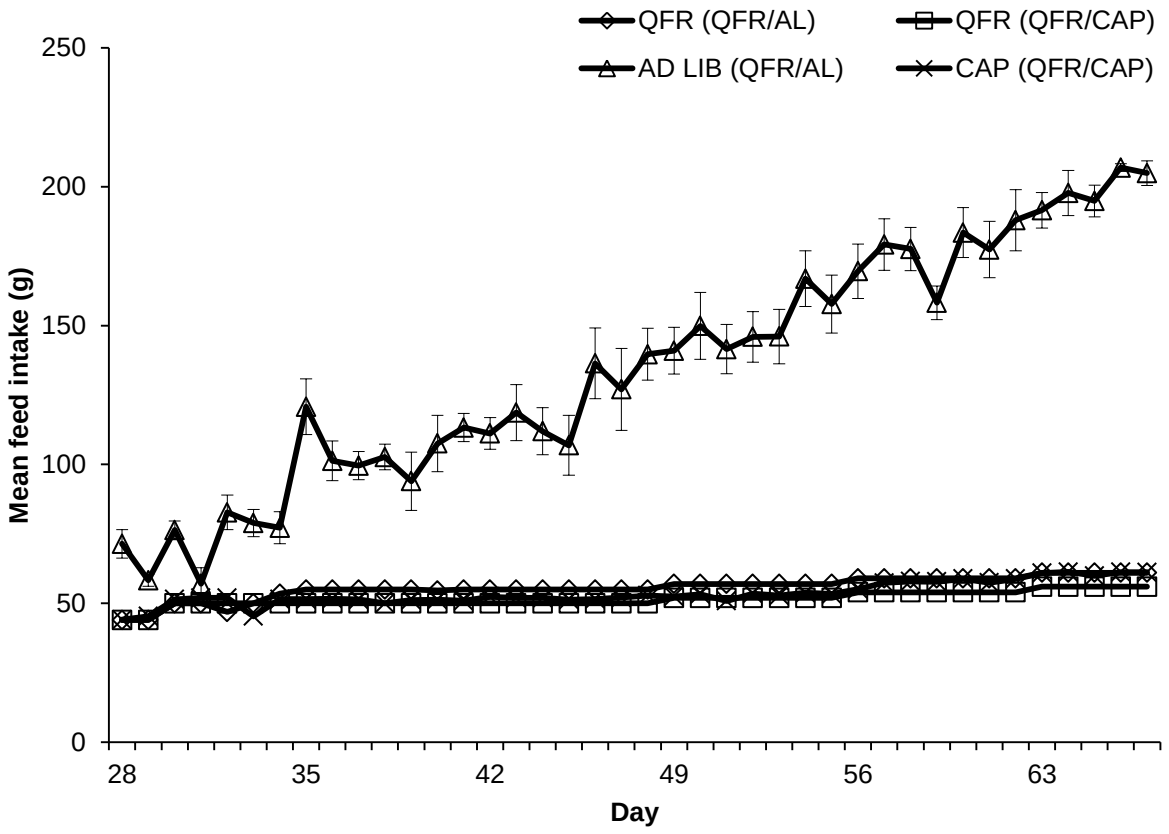
QFR/CAP birds grew at a similar rate to the commercial target (Figure 2), while QFR birds grew at a faster rate. This was to be expected as QFR/CAP birds were fed a similar quantity of basal diet as birds fed to commercial levels of feed restriction, while QFR/AL birds consumed considerably more feed on days when they were fed an *ad libitum* ration (Figure 3). The average consumption (± standard deviation) on *ad libitum* days was 58.3g (± 5.2g) (day 29) – 204.9g (±10.9g) (day 67).

Figure 2: The growth rate of the birds in treatment groups QFR/AL and QFR/CAP. The target growth rate for Ross 308 Broiler Breeders (fed to 5% production at 25 weeks) is also shown for comparison. Error bars are omitted as the S.E.M. for each group was too small to illustrate effectively. The S.E.M. for each of the time points shown was as follows: QFR/AL: 9.1g; 12.2g; 23.5g; 26.2g; 37.2g; 32.5g; 43.0g; QFR/CAP: 9.9g; 6.7g; 6.7g; 7.9g; 9.0g; 7.9g; 9.0g. Bird growth rate is shown to 70 days (this trial ended on day 67 but the birds remained on the same diet protocol as they were re-used for a further experiment).



Over the duration of training and testing the level of feed restriction of QFR/CAP birds was 22 - 24% (compared with birds of the same age) or 43 - 44% (compared to birds of similar bodyweight) of the QFR/AL birds *ad libitum* intake (range established from the first and last days of CPP training and resting, i.e. days 44 and 67 and based on the difference between the QFR ration for the QFR/CAP birds and the estimated daily intake of *ad libitum* fed broilers (Aviagen, 2007) for birds of a similar weight (age or bodyweight-matched respectively)).

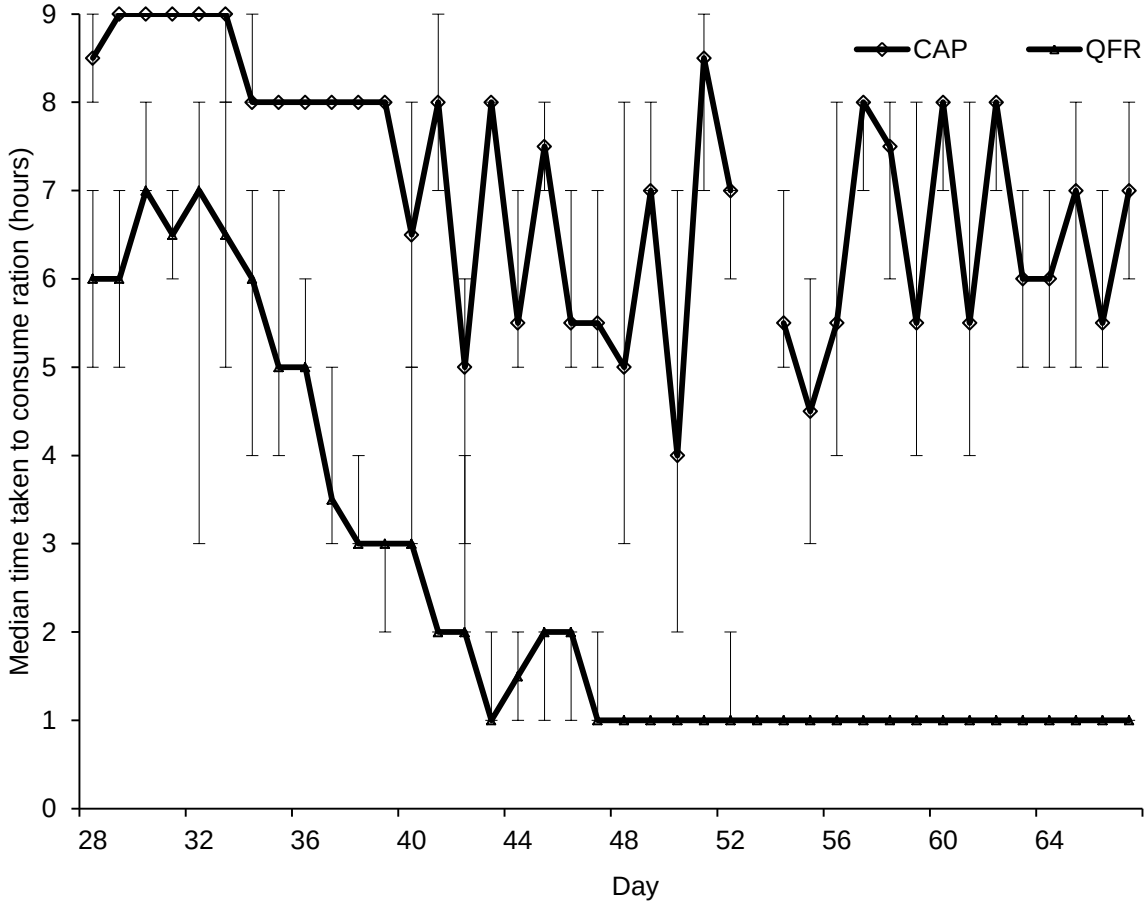
Figure 3: Daily feed intake of the diet options by the treatment group QFR/AL and QFR/CAP. The error bars indicate the daily S.E.M. and are shown only for the AL group. The mean daily S.E.M. for QFR intake for both the QFR/AL and QFR/CAP treatment groups was 0g. The mean daily S.E.M. for CAP intake was 1g.



5.5.2. Time taken to consume QFR or CAP ration by the QFR/CAP birds

As expected the CAP ration always took longer to consume than the QFR ration (Figure 4). However, there was considerable variation between birds in relation to the time taken to consume the CAP ration. The inter – day median time (with inter – quartile range shown) taken to consume the CAP ration across the period of CPP training and testing (day 44 – 67) was 6.2 (5.7 – 7.2) hours. However, intra – day there was considerable more variation apparent between birds.

Figure 4: The time taken to consume either the CAP or the QFR ration by the treatment group QFR/CAP. The Inter – quartile ranges are shown by the error bars. The dashed horizontal line represents the 8h cut off point. Birds failing to consume ≥ 95% of the daily ration by the 8h cut off were awarded 9h as a nominal value to aid graphical representation. There were no days on which birds failed to fully consume the QFR ration by 8h. The number of birds that failed to consume the full CAP ration by 8h are as follows: day 28: 3; day 29: 5; day 30: 5; day 31: 5; day 32: 3; day 33; day 37: 1; day 38: 1; day 41: 2; day 45: 1; day 51: 3; on all other days 0. The calcium propionate inclusion rate started at 3% and was increased to 4% on day 36 (38), 5% on day 41(43), 6% on day 45 (47), 7% on day 49 (51), 8% on day 57 (59), 9% on day 60 (62) and remained at 9% until the end of the study (day 67). Data was unavailable for CAP consumption on day 53.

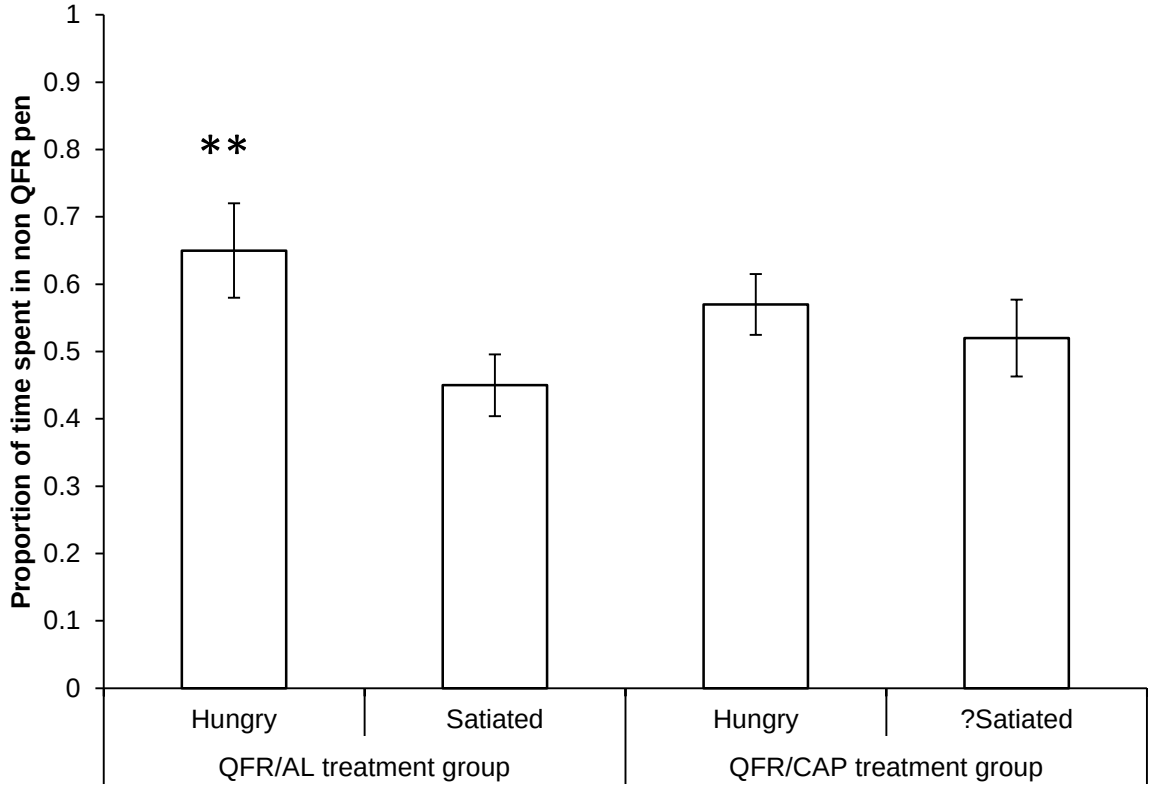


5.5.3. Proportion of time spent in each pen

The main variable of interest was the proportion of time the birds spent in either the pen associated with QFR or the pen associated with *ad libitum* access to feed (QFR/AL treatment group) or CAP (QFR/CAP treatment group). Here an effect of state at the time of testing ($F_{1,16} = 5.43$, P = 0.033) was observed. Post – hoc testing from 0.5 (no preference) indicated that only hungry (i.e. fed QFR on the day of testing) QFR/AL birds showed a significant preference ($T_{11}=3.27$, P = 0.007). This preference was for the pen associated with *ad libitum* access to feed (mean preference: 0.653; C.I. 0.550 – 0.757) (Figure 5). Ten out of 12

QFR/AL birds spent more time (shown as a proportion of total time tested) on the non – QFR pen when tested under conditions of feed restriction. The QFR/AL group mean of 0.45 (C.I. 0.29 – 0.60) did not differ significantly from 0.5 on days when QFR/AL birds were satiated (T_{11}=0.75, P = 0.467). No significant pen preferences were observed for QFR/CAP birds either when tested on QFR days (T_{11}=1.19, P = 0.259) or on CAP days (T_{11}=0.52, P = 0.611). The QFR/CAP group mean was 0.57 (C.I. 0.44 – 0.69) on QFR days and 0.52 (C.I. 0.42 – 0.62) on CAP days.

Figure 5: Effect of treatment and state at time of testing on proportion of time spent in the non – QFR associated pen. S.E.M. is indicated by the error bars. Only the QFR/AL treatment group, tested in a state of hunger (fed QFR on the day of testing) showed a significant preference (P < 0.01 level, denoted by **). The "?" prefix is present to indicate that the satiety levels of the birds in the QFR/CAP group, in comparison to the QFR/AL group, is an assumption.



No significant differences were found with pattern ($F_{1,16}$=2.3, P = 0.149) or side ($F_{1,16}$=0, P = 0.977) associated with QFR or test number (i.e. when tested after 12 and 24 days of training) ($F_{1,22}$=0, P = 0.946) in terms of proportion of time spent in each pen. Importantly, there was no effect of pen novelty with QFR/CAP birds showing no preference for either for or against the pen they had spent the last two days living in regardless of diet option fed on

the day of testing (Tested on CAP day: mean: 0.48; C.I. 0.38 – 0.58; T_{11}=-0.34, P = 0.739; Tested on QFR day: mean 0.57; C.I. 0.44 – 0.69; T_{11}=1.19, P = 0.259). QFR/AL birds tested under conditions of hunger (i.e. fed QFR on the day of testing) showed a significant preference for the novel pen. In this instance this corresponded with the preference for the pen associated with *ad libitum* access to feed, suggesting that the treatment effect was responsible, rather than a preference for novelty under only these circumstances. They did not show a preference when tested on days when fed *ad libitum* (mean: 0.45; C.I. 0.40 – 0.7; T_{11}=0.75, P = 0.467).

5.5.4. First pen entered

First pen entered did not reveal any significant preferences for either distinct environment. There was no effect of treatment ($F_{1,22}$=0, P = 1), state at time of testing ($F_{1,42}$=0.33, P = 0.566) or pattern ($F_{1,21.9}$=2.75, P = 0.111). Neither group first entered the pen associated with novelty (QFR/AL: χ^2=0.24, d.f. = 1, P > 0.05; QFR/CAP: χ^2=0.16, d.f. = 1; p > 0.05) or the pen associated with the non-QFR diet option (QFR/AL: χ^2= 0.10, P > 0.5; QFR/CAP: χ^2=0.10, P > 0.05) significantly more or less than 0.5. Furthermore, a side bias was no – longer evident in either treatment group (QFR/AL: χ^2=0.10, P > 0.05; QFR/CAP: χ^2=2.60, P > 0.05).

5.5.5. Number of pen changes

The median (inter – quartile range) number of changes between pens during tests was 14 (9 – 19.5) for the QFR/AL treatment group and 18 (11 – 25.5) for the QFR/CAP treatment group which was not statistically significant ($F_{1,22}$=2.47, P = 0.13). There was also no effect of diet option fed on day of testing ($F_{1,22}$=0.2, P = 0.661) and no interaction between treatment and diet option fed on day of testing ($F_{1,22}$=0.07, P = 0.793).

5.6. Discussion

The key significant findings from this study were that the QFR/AL birds expressed a preference for the pen associated with *ad libitum* feeding but only under conditions of deprivation (hunger) and the birds in the QFR/CAP group failed to demonstrate a preference for the environment associated with either diet option.

5.6.1. The QFR/AL birds' pen preferences

The finding that feed restricted broilers could learn a food – rewarded CPP under certain circumstances (the control group, i.e. QFR *vs.* AL feeding) but express it only when acutely feed restricted was unexpected. The failure of the QFR/CAP birds to show evidence of attraction to the novel pen suggested that the QFR/AL birds were not attracted to a pen due to its relative 'novelty'. Further, the lack of difference in pen changes between the two groups of birds or interaction with state at time of testing provided a crude indicator that the QFR/AL birds, when tested under conditions of hunger, had not simply picked a pen to forage in (anecdotally, the predominant activity) and then failed to move. Rather, they repeatedly returned to their favoured side. Thus, it seems that a state – dependent preference was being observed. This provided an interesting additional or alternative explanation for the birds' preference expression. It had been assumed that the birds would pick the pen associated with feeling more satiated because this is a positive affective state and birds would prefer to spend their time in a pen they associate with feeling 'good' (satiated) rather than in a pen they associate with feeling 'bad' (hungry). This is the basis for most CPP tests in pharmacological research (Tzschentke, 1998; Bardo and Bevins, 2000). However, Spiteri *et al.* (2000) found that morphine – conditioned rats spent less time active and more time in close association with the conditioned stimulus. By comparison, food – conditioned rats were more active and showed more exploratory behaviour. They concluded that rats given morphine had associated the environment with the post – affective state induced by morphine. By contrast, rats rewarded with food had learnt that the food – rewarded environment was a good place to find food which stimulated appetitive, food – seeking behaviour. This suggests that the QFR/AL birds in our study, when tested on days when hungry, perhaps selected the pen associated with *ad libitum* food supply not because they associated that environment with a more positive state but because they anticipated that they would be more likely to obtain food within this pen.

Few food rewarded – CPP studies have used a within – subject comparison between state of deprivation (e.g. hungry versus not hungry) to assess hunger – state – dependent preferences. Perks and Clifton (1997) trained food – deprived (to a bodyweight no less than 85% of *ad libitum* intake) but water – satiated rats to associate one environment with sweetened mash diet and another environment with sweetened water. Both rats were then tested under two different motivational states: thirst and hunger. They found a state – dependent preference: the rats preferred the pen associated with water when thirsty and *vice versa*. This indicated that the rats associated each distinct environment with resources of potential future value rather than post – consummatory affective state during training (although this latter association may also have occurred). Otherwise, the rats would have shown a preference for the mash – associated pen as they encountered this in a state of deprivation during training so its motivational value at the time of learning should have been higher than the sugar water. Further, the authors demonstrated that devaluing the sugar water post training of the CPP by pairing it with lithium chloride (in the home pen) reduces the strength of CPP expressed.

Where between – subject studies have been performed they have indicated that the pre – fed animal demonstrates either no CPP (Figlewicz *et al.,* 2001) or an attenuated CPP (Bechara and van der Kooy, 1992; Lepore *et al.,* 1995). Although some studies have found a food – rewarded CPP in non – deprived subjects (Papp, 1988; Papp, 1989; Papp *et al.,* 1991; Bechara and van der Kooy, 1992; Muscat *et al.,* 1992; Willner, *et al.,* 1994; Lepore *et al.,* 1995) the studies by Papp (1988, 1989) Papp *et al.* (1991), Muscat *et al.* (1992) and Willner *et al.* (1994) all adopted a methodology that included feed restriction throughout training. The rats are described as pre – fed before training but limited detail is available so it is difficult to determine how satiated the rats would have been before testing for CPP. It seems unlikely that the rats would have fully compensated for chronic feed restriction during training during the small interval between cessation of training and the CPP test (at most 24 hours). By contrast, our methodology in which broilers alternated every two days between feed restriction and *ad libitum* feed regimes probably allowed the birds to compensate to a degree as broilers can increase feed intake to near *ad libitum* levels on skip – a – day regimes (Dunnington, 1987). Thus our QFR/AL birds were expected to be satiated on days when given *ad libitum* access to feed prior to CPP testing.

Most food – rewarded CPP tasks train and test the animals under the same condition (Feed restriction: Guyon, *et al.,* 1993; Popik and Danysz, 1997; Chaperon, *et al.,* 1998; Spiteri *et al.,* 2000; Figlewicz *et al.,* 2001; Yonghui *et al.,* 2006; Zombeck *et al.,* 2008; Koizumi, *et al.,* 2009; *Ad libitum* access: Imaizumi *et al.,* 2000, 2001; Jarosz *et al.,* 2006; Dickson *et al.,*

2010; Matsumura *et al.*, 2010); therefore, it is not possible to clearly disentangle the effects of training (state – dependent learning) from testing (state – dependent preference). However, state – dependent preferences have been observed in food – and sucrose water – rewarded CPP tests. Naloxone (a dopamine receptor antagonist) (Jarosz *et al.*, 2006) and Naltrexone (an opioid receptor antagonist) (Delamater *et al.*, 2000) abolish food – and sucrose – conditioned CPPs respectively when injected before testing rats for CPP presence. In contrast, the dopamine receptor agonist, MK-801, both increases feed intake and potentiates expression of food – rewarded CPPs when administered pre – test (Yonghui *et al.*, 2006). Finally, Larson (2006) found a sucrose – water CPP was only expressed when rats were water – deprived prior to testing. These studies indicate that state at time of testing can affect the expression of food (or water) rewarded CPP. The current study supports these studies and indicates that the state of the animal during testing should be considered when designing CPP studies to determine feed preferences in feed restricted broilers.

However, environmental preferences have been observed in animals pre – fed prior to testing for a food – rewarded CPP. Papp (1988), Papp *et al.* (1991) and Spyraki *et al.* (1982) all trained under deprivation and fed prior to testing and found the rats demonstrated a CPP (but the effects of prior deprivation cannot be discounted as a motivator in these methodologies). No studies were found that trained under *ad libitum* conditions and tested under conditions of feed restriction (i.e. tested during a state of deprivation). Imaizumi *et al.* (2000, 2001), Jarosz *et al.* (2006), Matsumura *et al.* (2010) and Dickson *et al.* (2010) used rats fed *ad libitum* on chow outside the training situation. However, they trained a CPP in which the rewarding environment was associated with a higher value 'treat' food not available outside of the test situation. For example, corn oil (Imaizumi *et al.*, 2000, 2001) high sugar or high fat foods (Jarosz *et al.*, 2006), chocolate drops (Dickson *et al.*, 2010) or pre – training gastric infusions of glucose or corn oil paired with low nutritive foods within the apparatus (Matsumura *et al.*, 2010). By contrast, the less rewarding environment was associated with rat chow (except Imaizumi *et al.*, 2000, 2001, who used plain water). However, it is reasonable to assume any CPP that develops under these conditions develops as a consequence of a hedonic state induced by something other than the reduction of hunger. Thus, attainment of satiety (or, at least, reduction in hunger) is not a necessary condition of food – rewarded CPP learning. This has implications for the use of CPP to determine affective state in quantitative – or qualitatively – restricted broilers, both in how the test should be used, and what should be inferred from the demonstration of a food – rewarded CPP.

5.6.2. QFR/CAP birds failure to show a preference

The state – dependent preference observed in the QFR/AL group does not explain why the birds in the QFR/CAP group did not express a preference for one of the distinctive environments. This could have resulted from a failure to express a preference despite having learnt the relevant associations with environment or a failure to learn the task (and thus an inability to express any preference). These shall be discussed in turn.

5.6.2.1. Learnt the task but no preference exhibited?

One possibility for the failure to exhibit a preference is that the birds genuinely did not have a preference for either environment, perhaps because both distinct environments provided similar opportunities for the reduction of hunger. Alternatively, whilst differing across various dimensions (e.g. post – ingestion effects, sensory – led effects) the net effect in terms of affective state for the bird may have been perceived as similar between environments (e.g. the QFR environment may have offered a more palatable diet option than the CAP environment but resulted in higher levels of hunger than that experienced in the CAP environment). This study was not designed to investigate foraging decisions in hungry broilers. However, the finding that the QFR/AL birds expressed a preference for the pen associated with *ad libitum* feed access suggested that the birds selected the pen based on whether it was previously a good environment to forage in. Therefore, if a broiler's foraging behaviour is sensitive to time and it is able to recognise when food is likely to be available within an environment then our study design contained an inherent weakness. Namely, birds were tested during a period in which they had never received, or had access to, food. Most food – rewarded CPP studies are not closed economy and are likely to conduct their tests during a similar time of day to which the training took place. Therefore, the animal would enter the CPP apparatus expecting to find food within the chamber(s) that it had associated with food.

Both previous research by the authors and anecdotal observations in the current study suggested that the CAP option was aversive. Tolkamp *et al.* (2005) noted oral lesions (presumably associated with pain) when feed restricted broiler breeders were fed a mash diet which included 90g calcium propionate/ kg total feed. In the current study no gastrointestinal lesions were noted either during the study or at post – mortem. However, it was informally observed that some birds tried to escape the pen immediately upon being given their CAP ration (but never their QFR ration). This suggested that, whilst CAP was not associated with lesions (and the associated discomfort), it was not as favourably received by the birds as the

QFR ration. Thus, it was unexpected that a preference was not observed during the formal testing and this suggested that the diet option: distinct environment association had not been learnt.

5.6.2.2. A failure to learn the task?

A failure to learn the task appears counter intuitive given that QFR/AL birds did learn the task. However, several points can be made in favour of this interpretation. Firstly, QFR/AL birds expressed this state – dependent preference when hungry. There was *at least* one diet condition under which the QFR/CAP birds would have been hungry (QFR days). Therefore, there was *at least* one day during which the birds would have been in a state in which 'preference expression' (assuming one existed) could be anticipated. If birds were hungry on only one day or, at least *less hungry* on one day this would suggest that one diet was more satiating (and, presumably, more rewarding) and should have been preferred. Despite this, a preference was not expressed.

Secondly, if the effects of CAP on bird wellbeing (positive or negative) were not due to increased satiety, then the birds were trained and tested while fed a quantity of feed similar to commercial levels of feed restriction. Quantitative feed restriction is associated with behavioural and physiological indicators of stress in broiler breeders (e.g. Hocking *et al.,* 1993, 1996; de Jong *et al.,* 2002, 2003). Feed restriction is also associated with physiological changes such expression of, and levels of, certain nutritional – status – related compounds that may affect cognition (e.g. ghrelin, Diano *et al.,* 2006; synapsin proteins, Deng *et al.,* 2009). Buckley *et al.* (2011b) found feed restriction resulted in poorer performance on a food quantity discrimination task with birds fed to commercial levels of feed restriction failing to learn a food quantity discrimination task. Although most animals taught a food – rewarded conditioned place preference task are feed restricted, the level of restriction is less severe than the birds experienced (assuming that CAP had no or minimal satiating effects). Where reported, most studies restricted their animals (rats or mice) to somewhere within the range of 80 – 90% of *ad libitum fed* bodyweight (85 – 90%, Lepore *et al.,* 1995; 85%, Delamater *et al.,* 2000; 90%, Stuber *et al.,* 2002; 80 – 85%, Yonghui *et al.,* 2006) or circa 50% of expected *ad libitum* intake (Figlewicz *et al.,* 2001). This was less severe than the birds in this study. Further, for these animals, feed restriction began shortly before the study commenced. By contrast, the birds in the current study had experienced feed restriction from 14 days of age. Therefore, the birds in this study were considerably more feed restricted than in most other studies and this may have negatively affected learning.

Thirdly, high doses of propionate (sufficient to induce acidaemia) have been associated with later learning impairments in rats (Brusque, *et al.,* 1999; Pettenuzzo, *et al.,* 2002; Shultz, *et al.,* 2009; MacFabe, *et al.,* 2011). However, methodological differences limit the inferences that can be drawn. For example, those studies administered propionate subcutaneously (Brusque *et al.*, 1999; Pettenuzzo, *et al.,* 2002) or via intracerebroventricular injection (Schultz, *et al.,* 2009; MacFabe, *et al.,* 2011) whereas the birds in our study received CAP orally and could choose how much they ingested and over what time frame. Despite this, it cannot be discounted as a possible factor affecting the ability of the QFR/CAP birds to learn the CPP task.

Finally, extraneous stressors may have synergistically interacted with dietary stressors to prevent learning in the QFR/CAP birds. Chickens are a social species so social isolation can be expected to be stressful. Chronic social isolation negatively affected morphine or heroin rewarded CPP formation in rats (Kiyatkin and Belyi, 1991; Courdereau, *et al.,* 1997). The birds in the current study were individually housed during the day during the training and testing periods. This methodology was adopted due to concerns that testing the birds in pairs contributed to the lack of preferences exhibited in the Dixon *et al.* (pers. com) study. However, the long latency to consume the QFR ration by 4 week old QFR/CAP birds (data for QFR/AL birds was not recorded) immediately post – separation was atypical and unanticipated (they were consuming the daily ration in less than 40 minutes (unrecorded data) in the couple of days immediately preceding separation). The most reasonable explanation is this was primarily the effect of separation as increased vocalisation and attempts to access the other bird were evident. Further, the switch between the QFR ration and the CAP ration may have been experienced both as an uncontrollable and unpredictable environment condition (key components of many stressors, Wiepkema and Koolhaas, 1993) which would act as additional stressor. Exposure to chronic low level stressors has been demonstrated to abolish or attenuate either the learning and / or expression of a food – rewarded CPP task (Papp *et al.,* 1991; Cheeta *et al.,* 1994; Willner *et al.,* 1994). This may be particularly relevant in studies that use a closed economy design as the impact of environmental stressors can be protracted during CPP training.

5.6.3. Other methodological issues

In theory, the birds were tested during extinction (absence of food and food bowls). In practice, these may not have been true extinction conditions. During testing, the pens contained wood shavings. Informal observations made during this and other experiments by

the authors (unpublished observations) and Dixon (pers. comm.) indicate that the birds utilise these shavings extensively for foraging. It is inevitable that spilt food will be discovered reinforcing this behaviour. Further, in other experiments by the authors, birds consume wood shavings with considerable crop fill noted for some birds both whilst alive (author's own observations) and during post – mortem (Hocking, pers. comm.). Thus, shavings may have non-nutritive satiety – promoting properties (assuming that a full crop promotes satiety). In addition, shavings allow some natural behaviour to occur, occasionally yielding a nutritive morsel and distracting the birds' attention from the cues signifying the diet option to be found within this environment.

Regardless of the underlying potential value or impact of providing shavings it is suggested that it was an error to provide (or at least not control for) shavings during testing. De Jong *et al.* (2008) investigated CPP formation in pigs and found that pigs could form a CPP to an environment containing straw to forage in suggesting this was rewarding to pigs. Despite this, the performance of the QFR/AL birds indicates that, even with shavings provided during testing birds are able to demonstrate a CPP. This does not, however, account for the expected differential and / or relative value of shavings under the various feed options the birds encountered.

5.7. Conclusions and further research

It is concluded that there is some evidence that feed – restricted broilers can learn a food quantity associated CPP task. However, the presence of state – dependent preference expression means that it is essential to take this into consideration when designing such studies to maximise the chances of identifying a preference where one exists. Further, there was no evidence that CAP improves the welfare of feed restricted birds. Whilst a CPP was not observed, informal observations indicated that the birds did not like the CAP diet. Therefore, the more plausible interpretation is the birds failed to learn the task. However, this has not been shown by this current study and a genuine lack of preference cannot be discounted. Thus, further research should investigate the effect of both plane of hunger and calcium propionate on ability to learn a CPP task before adopting this technique more widely as a tool for the assessment of the welfare benefits of qualitatively-restricted diets.

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6. Experiment 4: The use of state-dependent learning to identify the relative satiating effects of quantitative and qualitative dietary restriction

6.1. Preamble

This chapter is currently in preparation for submission to a peer – reviewed journal.

Excerpts of this experiment have been presented at one external conference. The relevant references are:

Buckley, L. A., Sandilands, V., Hocking, P., Tolkamp, B. J. and D'Eath, R. B. (2012) The use of conditioned place preference to determine broiler preferences for quantitative or qualitative dietary restriction, *UK and Ireland Regional Meeting of the International Society for Applied Ethology*, 20th – 21st February, Shropshire, UK

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6.2. Abstract

The use of state-dependent learning (SDL) as a novel welfare assessment tool to quantify the effect of quantitative (QFR) or qualitative dietary restriction on broiler breeder hunger was evaluated in three experiments.

In each experiment, birds alternated every two days between two diet options with each diet option paired with a different coloured food reward. It was predicted that the reward associated with greatest hunger would be preferred in a subsequent choice test. Therefore, the QFR – associated reward would be preferred if the alternative diet option improved satiety. Each bird was tested twice (once per diet option fed on the test day).

In experiment 1 (pilot), birds alternated between QFR and *ad libitum* (AL) access (n = 4). During testing, birds preferred the QFR – associated reward during both tests (mean (± SD) proportional preference: 0.95 ± 0.08).

In experiment 2, birds alternated either between QFR and AL (QFR/AL group, n = 12) or QFR and QFR + calcium propionate (100g / kg total feed; QFR/CAP group, n = 12). Only the QFR/AL birds showed a preference (preference for QFR food reward, T_{12}=12, P = 0.006, test 1 only). However, differential reward intake during training by QFR/AL birds confounded the results in experiments 1 and 2.

In experiment 3, birds either alternated between QFR and AL (QFR/AL group, n = 8) or QFR and QFR + cellulose (100g / kg total feed). The food reward for the latter group was based on the cellulose diet (QFR/CEL (CEL) or the QFR diet (QFR/CEL (QFR) (both n = 8). All birds consumed all of the food reward on all training days. During testing, no reward preferences were found.

It was concluded that any SDL – derived preferences observed were an artefact. Thus, conclusions about any satiating effects of qualitative dietary restriction were not possible.

6.3. Introduction

State – dependent learning (SDL) is the phenomenon by which an animal shows a preference for something based on the context in which it originally encountered it. An animal that experiences a stimulus linked to a food reward when in a state of high deprivation and another stimulus linked to an identical food reward when in a state of low deprivation will often show a preference for the stimulus associated with a state of high deprivation (Pompilio and Kacelnik, 2005). Furthermore, this preference has been shown to be independent of the animal's current state at the time of the two – way preference testing (e.g. Kurtz and Jarka, 1968; Kacelnick and Marsh, 2001). A state of high deprivation can be induced external to the training situation by food restriction (Revusky, 1967; Kurtz and Jarka, 1968; Capaldi *et al.*, 1994; Pompilio *et al.*, 2006; Vasconcelos and Urcuioli, 2008), by making the animal work hard to access the food reward within the training situation (Clement *et al.*, 2000; Kacelnick and Marsh, 2001; Friedrich and Zentall, 2004; Gipson *et al.*, 2009) or by making the animal wait longer to access the reward (Pompilio and Kacelnik, 2005). These seemingly irrational preferences are thought to occur because the animal values the same reward differently dependent on its value to the animal at the time that it originally encountered it (Pompilio and Kacelnik, 2005). This may occur due to the increased contrast between hedonic states before and after receiving the food reward in a state of high deprivation during training relative to that experienced when in a state of low deprivation (Clement *et al.*, 2000) and / or due to a perceptual distortion (Pompilio *et al.*, 2006). It has been observed in a wide range of species (fish, Aw *et al.*, 2009; locusts, Pompilio *et al.*, 2006; pigeons, Friedrich *et al.*, 2004; Gipson *et al.*, 2009, rats, Kurtz and Jarka, 1968; Capaldi *et al.*, 1991; Capaldi *et al.*, 1994; and starlings, Marsh *et al.*, 2004; Pompilio and Kacelnik, 2005) indicating that it is a robust phenomenon. This has led researchers to conclude that it must be evolutionarily beneficial or rational in the natural environment (Pompilio *et al.*, 2006) despite it leading to irrational preferences in the laboratory setting.

The phenomenon of preferences caused by SDL has not thus far been applied to animal welfare assessment. It is proposed here to use SDL as a novel welfare assessment tool to investigate the relative satiating effects of quantitative and qualitative dietary restriction in the management of feed restricted broiler breeders. The studies described in this paper aim to test whether qualitative dietary restriction can induce a (relatively) 'low deprivation' state during learning, contrasting with the presumed high deprivation state of quantitative food

restriction (QFR). If so, the prediction is that this would be revealed by a later preference for the stimulus associated with the quantitative restriction. Feed restriction is a widely recognised welfare problem for fast – growing broiler breeders (de Jong *et al.*, 2003). Experimentally researchers have tried to improve welfare by adjusting the quality of the diet by either reducing the energy density, adding appetite suppressants or by a combination of both approaches (e.g. Rozenboim *et al.*, 1999; Savory and Lariviere, 2000; Nielsen *et al.*, 2003; Hocking *et al.*, 2004; Sandilands *et al.*, 2005, 2006; Nielsen *et al.*, 2011). However, whilst these are successful at increasing time taken to consume the ration, it is not clear whether these diets achieve this by improving satiety (a positive affective state) in the broiler breeder (D'Eath *et al.*, 2009). Direct choice test methodologies in which the broiler breeder chooses between either qualitative feed restriction or QFR have so far not proved successful (Buckley et al 2011a). Whilst this may be because hungry birds find it more difficult to learn food quality discrimination tasks (Buckley et al 2011b) it is possible that some other factor affected the lack of preference. Further, choice tests may not actually be measuring preferences determined by altered states of satiety. Thus, there is a need for alternative approaches to identify which, if any, diet option is more satiating.

State of deprivation is defined in the following way: high deprivation is the state of hunger experienced by birds fed to commercial levels of QFR. Low deprivation is the state of satiety experienced by birds fed on an *ad libitum* basis. The aim of the following three experiments was to use SDL to identify whether diets adjusted with either an appetite suppressant (calcium propionate) or a bulky non – nutritive filler (cellulose) were more satiating than conventional QFR. The first experiment was designed to validate an SDL methodology using birds reared on an alternating schedule of QFR and *ad libitum* feeding. The second and third experiments were designed to use SDL to compare the satiating effects of calcium propionate (CAP) and cellulose (CEL) enriched diets. A validation group based upon experiment one was also used in experiments 2 and 3 as the methodology used varied slightly between experiments. A comparison of the total feed reward intake by birds tested on days when they had been fed QFR and days when they were fed the alternative diet was also undertaken. Finally, a two – pan test was carried out in experiment three to investigate hungry broiler breeder preferences for QFR or CEL after a period of time to learn about the relative satiating effects of each diet.

SDL Experiment one:

6.4. *Introduction*

The purpose of the first experiment was to identify whether the phenomenon of SDL could be reproduced in broilers, as a model for broiler breeders, alternating between a state of high deprivation and low deprivation in respect of feeding regime. The ultimate purpose was to validate a methodology that could be used as a 'probe of hunger state' when comparing the relative states of deprivation induced by QFR and qualitative dietary restriction.

6.5. Hypotheses

It was hypothesised that broilers would value an identical (but distinctly marked) food reward differently dependent on the state of deprivation that the bird was in when it first encountered the food reward. It was predicted that birds would prefer the food reward that they encountered under conditions of feed restriction (high deprivation) more than a food reward they encountered under conditions of *ad libitum* feeding (low deprivation). Furthermore, it was predicted that this preference would be independent of the state of deprivation at the time of testing. Finally, it was hypothesised that the total quantity of food consumed during testing would be affected by the state of deprivation at the time of testing with birds fed *ad libitum* on the day of testing consuming less food than birds fed QFR on the day of testing.

6.6. Methodology

6.6.1. Subjects and treatment groups

This study used four broilers (as a more readily available model for broiler breeders) aged 28 days. As a pilot study, it was intended to use six birds (all spares from another study) but two birds were euthanised before the start of the start of training and testing for poor growth despite supplementary feeding. All four birds followed the same dietary regime and acted as their own control.

Prior to this study the birds had been group reared on a 14:10 h light: dark schedule (day 1 – 28) and spot-brooded (day 1: 31˚C, reduced gradually to 21˚C on day 21 and maintained at this temperature thereafter). The birds were fed a commercial starter chick crumb (Farmgate, BOCM Pauls Ltd., Ipswich, Suffolk, UK) *ad libitum* from 1 – 14 days and thereafter feed restricted in line with the recommended daily feed requirements for broiler breeders (Aviagen, 2007). The mean (± standard deviation) bodyweight of the birds at the beginning of the study at 28 days of age was 551.3g (± 92.3g) which was approximately 20% heavier than the target bodyweight for broiler breeders at 28 days (440g). They had no previous experimental history.

6.6.2. Housing and husbandry

Each bird was individually housed in a floor pen (1.05m × 0.45m) with visual access to one other conspecific through a mesh divider. A solid barrier by the feeding area prevented each bird from seeing what food the other bird was eating. Each pen contained wood shavings and a perch. Birds were fed once daily at 09:00h and any food remaining was removed at 16:00h, weighed and the birds' daily feed intake recorded. Water was available *ad libitum*. Birds were maintained on a 9:15h light : dark schedule and a room temperature of 21 - 23˚C throughout the study.

Each bird alternated every other day between being fed a quantity of feed equivalent to commercial feed restriction (QFR) and *ad libitum* (AL) access to the same diet between 09:00 – 16:00h. Half the birds started this feeding regime on QFR and half the birds on AL. This schedule was maintained throughout the study, from day 28 until day 65 with the exception of the two days of SDL preference testing and the washout day (described below).

6.6.3. Feed and nutrition

The main diet was a custom – made grower mash (Target Feeds, Whitchurch, Shropshire, UK) suitable for broiler breeders from 28 days of age. The diet contained 165 g/kg crude protein and metabolisable energy (ME) of 12.1MJ / kg feed.

The food rewards were comprised of the commercial starter chick crumb that the birds were initially reared on (Farmgate, BOCM Pauls Ltd., Ipswich, Suffolk, UK). This diet was used as it had a higher protein and energy density than the grower mash and was expected to be attractive to the birds irrespective of their level of deprivation. The reward diet was either stained red or green using food colouring (Silverspoon, Cambridgeshire, UK). The food rewards were stained by mixing 10 ml of food colouring diluted with 20 ml of water with each 100 g of feed. The feed was then dried to a similar consistency as the original feed by drying in a warm oven (40˚C) for approximately 60 minutes.

6.6.4. SDL protocol

6.6.4.1. Training

Training started when the birds were 47 days old. The birds had any feed remaining removed at 16:00h (in practice this was only the birds being fed AL). All birds then received a 15 g food reward that was either stained red or green. Half the birds received the red reward on days when they had been fed QFR and the green reward on days when they had been fed AL (and the remaining birds vice versa). Birds were given 2h to consume the ration. Any ration left was weighed, discarded and each bird's intake recorded.

6.6.4.2. Testing

After eight days of training (i.e. four days per food reward: diet option pairing) birds were tested on day 55 for the presence of a SDL preference. Birds were fed the same diet option that they had received on day 54 (e.g. AL) on the day of testing. Birds were then given a 'washout' day in which they received no food reward and received the diet option that they did not receive on day 55. Birds were then given another either eight days of training (during which they alternated every two days between diet options) and then tested again on day 65.

Each bird was tested twice for the presence of a SDL preference: once on a day when they had been fed QFR and once on a day when they had been fed AL. The order of testing and diet colour associated with QFR was counterbalanced between birds with half tested first on a QFR day and then on an AL day and the other half in the reverse order.

Testing took place within the home pen between 16:00h – 16:30h. Each bird was offered a bowl of red food reward (70 g) and a bowl of green food reward (70 g) and allowed free access to each bowl for three minutes. The first bowl approached was recorded, and time (seconds) spent eating the red food, the green food or not eating was recorded. After three minutes the bowls were removed, the contents weighed and the intake of each was recorded.

6.6.5. Statistical analysis

The sample size was too small for meaningful statistical analysis. Therefore, the results are presented using descriptive statistics and individual bird performance only.

6.6.6. Ethical note

This study (and experiment two) were carried out under Home Office license and were approved by SAC and the Roslin Institute's animal ethics committees. Experiment three was approved by the Harper Adams University College ethics committee and was not carried out under Home Office license but was approved by the local Home Office inspector. Although individually housed, the birds either had visual access to another bird (experiment one and three) or were pair housed overnight (experiment two). The space allowance exceeded the Home Office minimum guidelines at all time. The birds were provided with a perch (experiment one and two) and some wood shavings to facilitate natural behaviours (all experiments). Water was available *ad libitum* for all experiments. The alternating feed schedule ensured that the birds' level of feed restriction was less (birds fed QFR versus AL, all experiments) or at least no more severe than under commercial practice (birds fed QFR versus the alternative diet, experiments two and three) and no health problems were expected or observed as a consequence of adding the food colouring. The birds in experiments one and two were euthanased by barbiturate overdose and the birds in experiment three were re – homed at the end of the study.

6.7. Results

6.7.1. Daily food consumption during training and testing

The birds always fully consumed the daily food ration when the diet option was QFR. The mean (± standard deviation, SD) daily take of QFR over the period of training and testing was 42.6 g (± 2.4 g). The birds consumed considerably more on days when fed AL and consumed a mean (± SD) daily intake of 114.1 g (±25.3 g) over the same period.

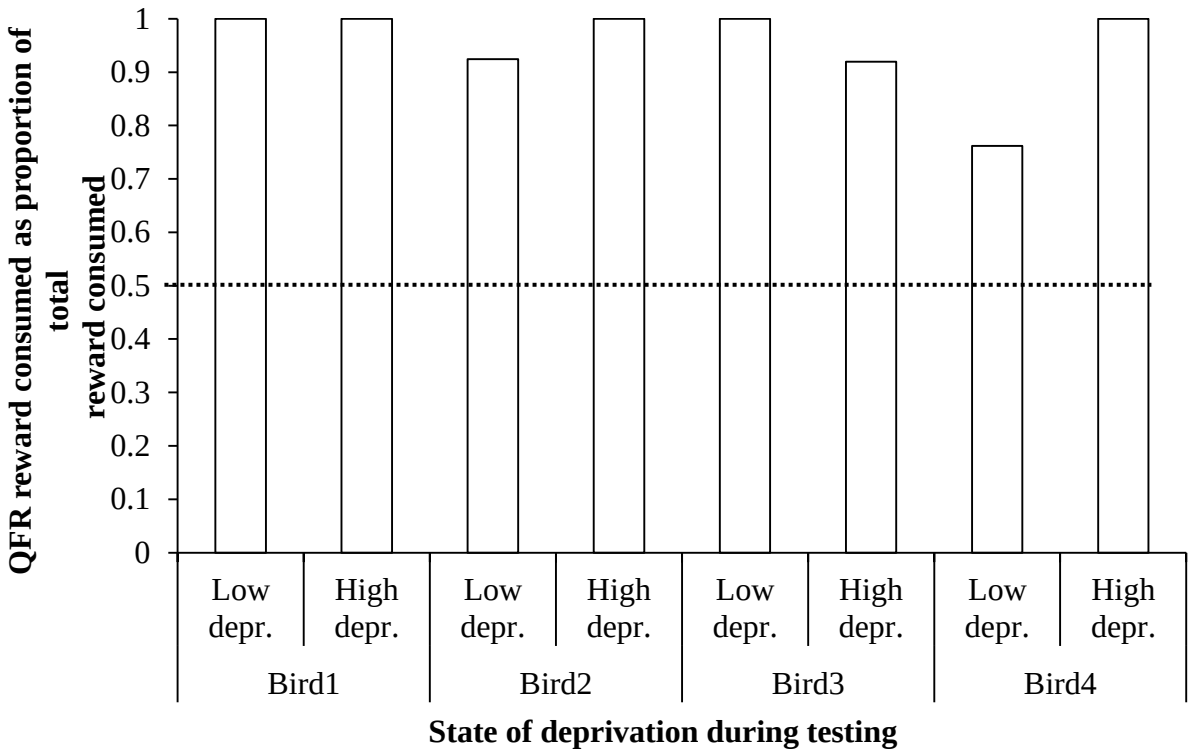
6.7.2. Food reward consumption during SDL training

The birds always fully consumed the food reward (15 g/day) on days when they were fed the QFR option. However, the birds failed to fully consume the food reward on the first three days when fed the AL option. Four birds failed to fully consume the food reward on day one, three birds on day two, and one bird on day three of AL – food reward training. All birds fully consumed the AL food reward on subsequent days. The mean (± SD) individual birds' cumulative daily intake of each food reward by the first SDL test on day nine of training and testing was 60 g (± 0 g) for the QFR-associated food reward and 40.2 g (± 13.3 g) for the AL – associated food reward. The mean (± SD) individual birds' cumulative intake of each food reward (including the food reward consumed during the first SDL test) by the start of the second SDL test was: QFR – associated food reward: 148.4 g (± 5.9 g); AL – associated food reward: 101.5 g (± 16.7 g).

6.7.3. SDL preferences

All four birds showed a preference for the food reward associated with high deprivation (i.e. fed QFR on the day of testing; see figure one). This preference was very similar across both tests which indicated that the birds' state of deprivation at the time of testing did not influence the direction of the preference.

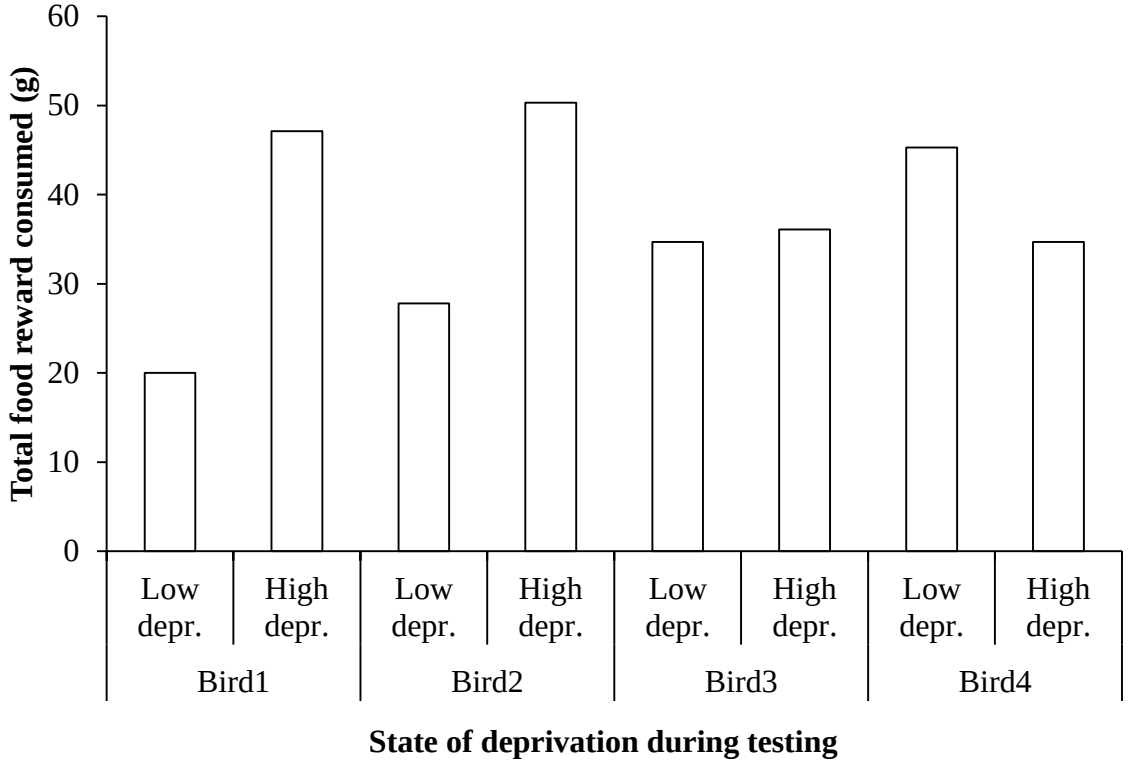
Figure 1: Experiment 1: The effect of state of deprivation on birds' preference for the QFR food reward. The QFR food reward consumption is expressed as a proportion of total food reward (red and green) consumed during the three minute test. The dashed line represents the 0.5 point of no preference.



6.7.4. Effect of state on food consumption during each test

The mean (±SD) intake of the birds was 32 g (± 10.7 g) when tested on a day when they were fed AL and 42.1 g (± 7.8 g) when tested on a day when they were fed QFR. The individual intake of each bird can be seen in figure two.

Figure 2: Experiment 1: Total intake of food reward (red plus green) by each bird under low (*ad libitum* feed) and high (QFR) deprivation.



6.7.5. Anecdotal observations

Although this was not formally measured, it was observed that, during testing, consumption of the food reward (both colours) were accompanied by exaggerated gaping of the beak and pronounced 'neck ripples' during swallowing.

6.8. Discussion

Although the sample size was small, the experiment appeared to have validated the use of SDL approaches in hungry broilers, using QFR and AL to induce high and low deprivation states. However, the fact that the birds failed to fully consume the food reward associated with low deprivation (fed AL on that day) during the initial stages of training represented a confounding factor. It could not be certain whether the apparent preference was genuinely due to the birds valuing each food reward differently, dependent on their state of deprivation at the time of encountering the food reward, or due to the differential quantities consumed affecting associative strength or preference in some way. Therefore, although SDL appeared to have potential, further experiments must ensure that all the food reward is consumed under both states of deprivation in order to have confidence in the meaning of any SDL preference observed. Feed intake during the test also appeared to be affected by the state of deprivation at the time of testing. However, the individual bird data suggested that there was overlap between the intake of the birds tested under conditions of high and low deprivation. Therefore, if this parameter is to be assessed it may be more appropriate to increase the testing time to allow differences in intake to become more apparent.

SDL Experiment two:

6.9. Introduction

Experiment one suggested that SDL could occur in broilers trained in states of high and low food deprivation (using QFR and ad libitum feeding respectively), which indicated that it might be a potentially useful tool to investigate whether qualitative dietary restriction improves satiety in hungry broiler breeders. Experiment two was designed to investigate this further by comparing the preferences of broilers reared on an alternating schedule between QFR and a diet quality – adjusted by the addition of calcium propionate (CAP).

CAP is a feed additive that has previously been shown to reduce feed intake (relative to *ad libitum* intake of an otherwise similar basal feed) (Sandilands *et al.,* 2005). The mechanism of appetite suppression is unclear but thought to involve various metabolic pathways post – absorption associated with satiety (Arora *et al.,* 2011), although CAP has also been linked to gastrointestinal inflammation (Bolton and Dewar, 1964; Al-lahham *et al.,* 2003; Tolkamp *et al.,* 2005), nausea (Frost *et al.,* 2003) and reduced feed palatability (Darzi *et al.,* 2011). These may all reduce intake without improving the state of deprivation. The birds used in the current study had just been used in a conditioned place preference study aimed at the determination of the welfare effects of CAP on broilers (Buckley *et al.,* In press). However, they failed to show a preference for either an environment associated with QFR or an environment associated with CAP. It was unclear whether this was due to a lack of preference or a failure to learn the task (Buckley *et al.,* 2011a). Thus, there is a need for additional approaches to try and quantify the effects of calcium propionate on the state of deprivation and it was decided to use SDL to further investigate this.

6.10. Hypotheses

The primary hypothesis under investigation is that broilers will show a SDL derived preference for a food reward fed on days when fed QFR (high deprivation) over a food reward received on days when fed when the same quantity of QFR but quality – adjusted to contain the appetite suppressant calcium propionate (CAP; unknown level of deprivation). This hypothesis assumes that CAP induces a state of satiety similar to the 'low deprivation' treatment in experiment one. Alternatively, no preference, or a preference for the QFR training stimulus would indicate that there was no such satiety effect and birds remained in a state of high deprivation. A control group that had to choose between food rewards associated with high deprivation (QFR) and low deprivation (*ad libitum* feed) as in experiment one was

also included. Finally, it was hypothesised that total food reward intake would be affected by the state at the time of testing. It was expected that for control birds (QFR/AL) total food reward intake would be lower on days when birds had been fed *ad libitum* than on days when they had been fed QFR. Similarly for the QFR/CAP treatment it was predicted that if CAP was associated with a lower state of deprivation then birds would show a reduced intake of the food reward when tested on days when they had been fed CAP in comparison to being tested on a day when they had been fed QFR.

6.11. Methodology

6.11.1. Subjects and treatment groups

24 Ross 308 broilers (as a model for broiler breeders) were used for this study. These birds had been previously used in a related behaviour study (Buckley *et al.*, In press; chapter five) and the housing arrangements and experimental regime are described more fully there. These birds were admitted to this study aged 69 days. They remained in the same treatment groups that they were allocated to in the previous experiment rather than redistributing to balance for the differing weight. Birds were maintained in these groups as they were accustomed to this feeding regime and controlling for previous experimental history by balancing for bodyweight raised both experimental problems (small sample size) and practical problems (quantities to feed the different sized birds within treatment group). In brief these two groups were: 1) QFR/AL (n = 12) and 2) QFR/CAP (n = 12). The experimental variable was the diet regime the birds were given and in all other respects the birds were treated equally. The mean (± SD) body weight of the birds at the start of this experiment was: QFR/AL group: 1991.8 g (± 148.8 g); QFR/CAP group: 1160.7 g (± 31.0 g).

6.11.2. Housing and husbandry

The birds experienced the same lighting and temperature regime as in experiment one. During the day they were housed in similar pens with the exception that they had no visual access to another bird (due to the constraints of the previous experiment). Instead, they were pair – housed overnight with a bird from the same treatment group. Water was provided *ad libitum* throughout the study; although, problems with water delivery during the second SDL training period meant that the birds were occasionally without water for short periods of time (1 – 2 hours day) for approximately three of the training days.

Each bird alternated every other day between being fed a quantity of feed equivalent to commercial feed restriction (QFR) and an alternative diet option. For QFR/AL birds the alternative diet option was *ad libitum* access to the basal diet between 09:00 – 15:00h. For the QFR/CAP birds the alternative diet option was the QFR ration with the addition of calcium propionate (CAP). Any food remaining at 15:00h was removed, weighed and the birds' intake recorded. In practice, this only applied to birds being fed on an *ad libitum* ration. This schedule was maintained throughout the study with the exception of the two days of SDL preference testing and the washout day (described below).

6.11.3. Feed and nutrition

The basal diet was identical to that described in experiment one. Calcium propionate (Propimpex® CA powder, Impextraco, Germany) was added to the CAP diet at an inclusion rate of 10 g CAP / kg total feed. The red and green food rewards were the same as those described in experiment one.

6.11.4. SDL protocol

6.11.4.1. Training

The training phase was identical to that described in experiment one with the following exceptions. The birds were given 10 g of food reward (instead of 15 g) and they were offered this reward at 15:00h and given three hours to consume it. The quantity of food reward offered was decreased to try to ensure that the birds consumed equal amounts of each food reward prior to the first test as this had been a problem in experiment one.

6.11.4.2. Testing

The testing phase was identical to that described in experiment one with the following exceptions. The birds were given 100 g (instead of 70 g) of each of the different coloured food rewards, the test lasted five minutes per bird (instead of three minutes) and testing took place between 15:00 – 18:00h. The time allowed for the birds to consume the food reward during the test was increased to five minutes to improve any contrast between quantity of food reward consumed when in a state of high deprivation (fed QFR option that day) and when in either a state of low deprivation (fed AL that day) or a state of unknown deprivation (fed CAP that day) as the results of experiment one suggested that three minutes was insufficient for this purpose.

6.11.5. Statistical analysis

All statistical analyses were carried out using Genstat 13th Edition (VSN International, Hemel Hempstead, UK). The main treatment effects (for both proportions of food reward consumed and quantity of food reward consumed) were analysed using a repeated measures REML on untransformed data (analysis of the residuals indicated that these were normally distributed). Comparisons with 0.5 were carried out on untransformed proportion data using a One Sample sign test and a Wilcoxon matched – pairs test as the data could not be normalised despite various transformations. Inter – group and intra – group comparisons of the quantity of feed consumed were carried out using a student's T – test using untransformed data.

6.12. Results

6.12.1. Daily food consumption during the period of SDL training and testing

All birds fully consumed the daily allocation of feed when QFR was the diet option offered. The mean (± SD) daily intake on QFR days was: QFR/AL birds: 64.8 g (±1.8 g); QFR/CAP birds: 59.8 g (±1.8 g). The difference in consumption reflects differences in the quantity of QFR offered to each treatment due to bird body weights. The QFR/AL birds grew larger than QFR/CAP birds and required an increased ration to maintain growth on days when fed QFR as heavier birds require more feed for maintenance requirements.

As expected, on days when the birds were offered the alternative diet the QFR/AL birds consumed considerably more food. The mean (± SD) daily intake when offered AL over the duration of training and testing was 210.6 g (±19.8 g). For QFR/CAP birds, the mean (± standard deviation) daily intake on days when fed CAP was 64.0 g ± 2.3 g with the full ration consumed by all birds on most of the days.

6.12.2. Food reward consumed on days of SDL training

All birds consumed the full food reward on all days when fed QFR (10 g /day). The QFR/CAP birds also always consumed the full food reward on days when fed CAP (10 g /day). However, all of the QFR/AL birds failed to fully consume the food reward associated with AL on the first two days of training.

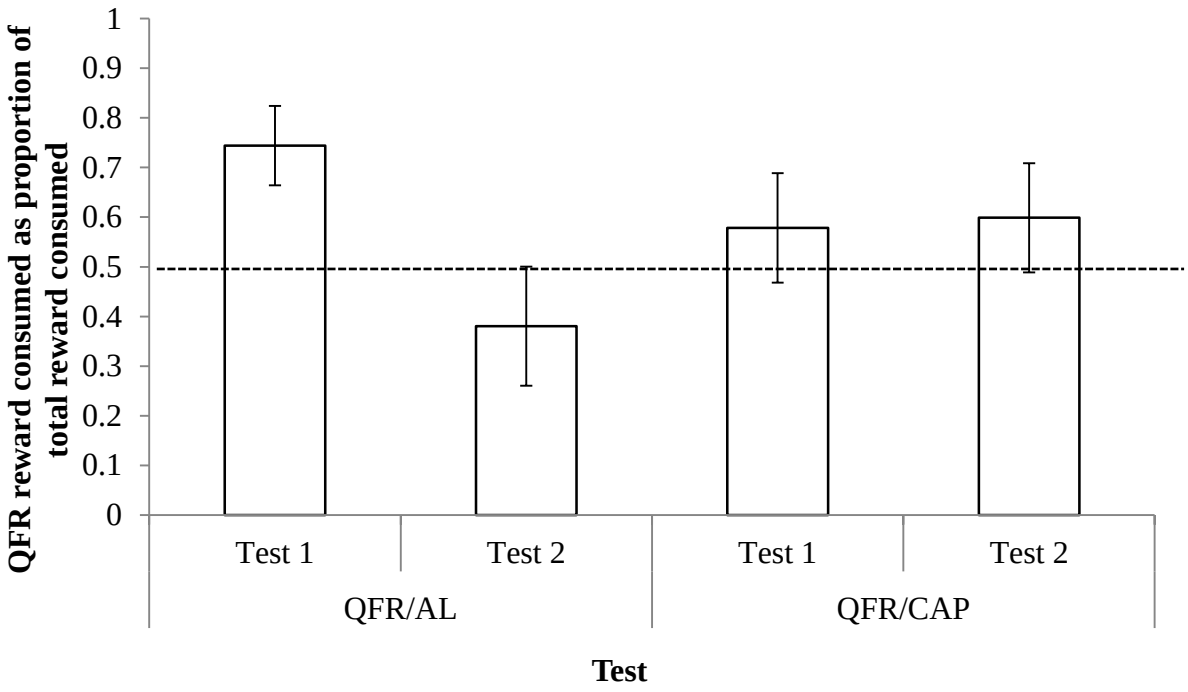
For the QFR/AL birds, the mean (standard deviation) cumulative intake (over four days per food reward) by each individual bird of the food reward associated with QFR by the start of the first SDL test was 40 g (± 0.0 g) and for the food reward associated with AL it was 33.8 g (± 5.9 g). By the start of the second test (eight training days per food reward plus the quantity consumed during the first test) the mean cumulative intake (including food reward consumed during the first SDL test was 111.6 g (±15.4 g; QFR – associated reward) and 83.2 g (± 4.7 g; AL – associated food reward).

For the QFR/CAP birds the mean (±SD) cumulative intakes were as follows. By the start of the first SDL: 40 g (± 0.0g) (for both the QFR – associated and the CAP – associated reward). By the start of the second SDL: 103.8 g (± 16.3 g) (QFR associated food reward) and 97.9 g (± 5.9 g) CAP associated food reward).

6.12.3. SDL preference test

There was no effect of treatment ($F_{1,20.0}$=0.04, P = 0.843) or diet option fed on the day of testing ($F_{1,20.0}$=0.15, P = 0.704) on the proportion of food reward associated with QFR. However, there was an effect of test number ($F_{1,20.0}$=4.35, P = 0.05) and an interaction between test number and treatment ($F_{1,20.0}$=5.45, P = 0.030). An examination of the least significant differences (5% level) indicated that birds in the QFR/AL (but not the QFR/CAP) groups consumed significantly more food reward associated with QFR during test one than in test two. This was significantly different from the 0.5 chance level only during test two (T_{12}=12, P = 0.006) with 11 out of 12 birds consuming more of the food reward when on QFR than when on AL in test one. In test two, 8 out of 12 QFR/AL birds consumed more food reward when on AL than when on QFR (see figure three).

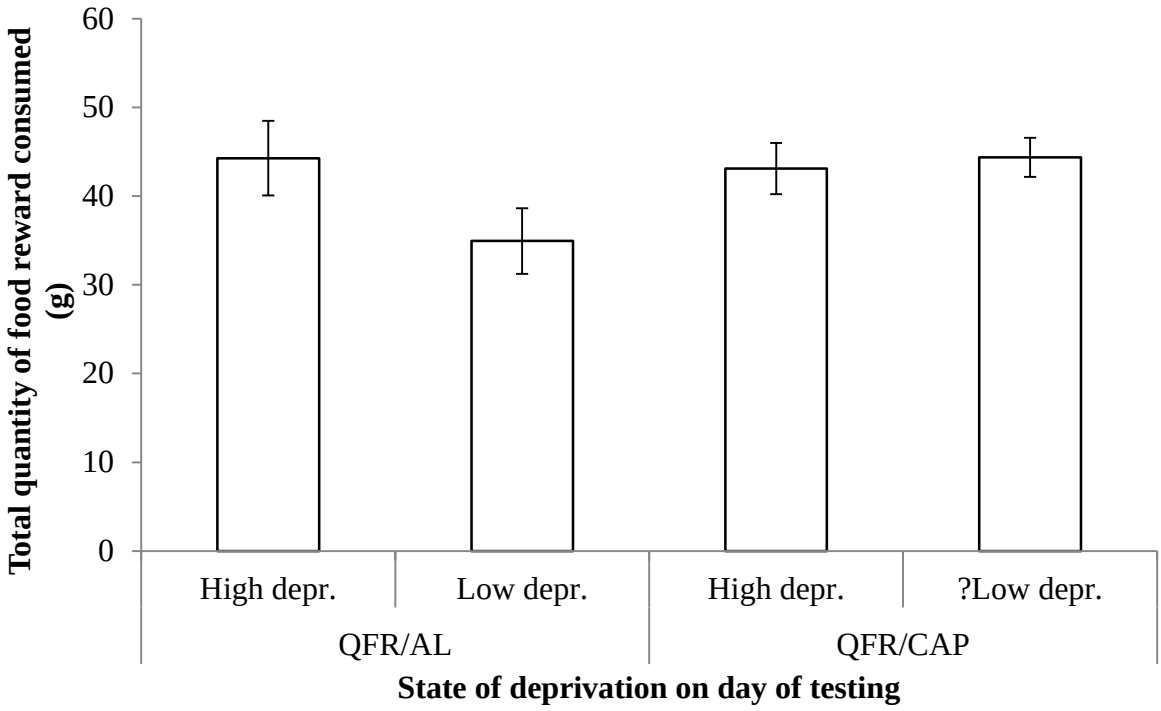
Figure 3: Experiment 2: The effect of test number and treatment group on QFR – associated food reward consumption. A significant preference for the QFR – associated food reward was observed for QFR/AL birds in test one only.



6.12.4. Total quantity of food consumed in each test

There was an effect of hunger state ($F_{1,20.0}$=4.82, P = 0.040) but not treatment ($F_{1,20.0}$=1.05, P = 0.318) or test number ($F_{1,20.0}$=0.15, P = 0.704) on the total quantity of the food reward consumed. Only birds in the QFR/AL group consumed significantly more food when tested on days that they had been fed QFR than on days when they had been fed the alternative diet option (in their case AL) (based on the least significant differences at the 5% level) (See figure four).

Figure 4: Experiment 2: The effect of treatment group and diet option fed on day of testing (state of deprivation) on total quantity of food reward consumed during the five minute SDL test. A significant difference between consumption of the food reward was only observed for birds in the QFR/AL group. The prefix "?" is used to denote that, in contrast to the QFR/AL group, a state of relatively low deprivation when fed CAP is only an assumption for the birds in the QFR/CAP group.



6.12.5. Anecdotal observations

Again, it was observed that the birds demonstrated exaggerated gaping behaviour and neck ripples during swallowing whilst consuming the food reward.

6.13. Discussion

The birds in the control group (QFR/AL) showed a SDL preference in test one but not in test two. This finding was problematic to interpret. Unfortunately, despite a reduced quantity of food reward offered during training and the increased time available to consume the food reward before its removal (at the end of the light period), the birds still failed to fully consume the food reward offered on days when fed AL. Thus, a confounding factor remained between the quantity of food reward consumed and the direction of the preference observed in test one.

The fact that this preference did not remain in test two is puzzling and it is not clear why this should be the case. It is possible that the problems with water supply altered the relative affective (i.e. feeling thirsty) or physiological state (dehydration) of the chickens in the QFR/AL group. Although problems with water did not appear to reduce feed consumption (suggesting that water supply was adequate for the physiological needs of the birds – feed restricted broiler breeders are well known to consume excess water), it cannot be ruled out that this influenced the relative palatability of a food reward that may have been perceived as aversively dry and hard in texture. Alternatively, the extra quantity of food consumed during this test may have conditioned an aversion to that food reward in some birds. Binge – feeding is associated with an aversion to the food binged on in rats (Hertel and Eikelboom, 2010). However, the quantities of feed consumed during the test were not that dissimilar to those consumed during experiment one and the strength or direction of preference was not affected by consumption in test one of the first experiment. Finally, it cannot be ruled out that the lack of preference in test two shown by QFR/AL birds was simply a consequence of a possible initial neophobia to coloured feed being overcome and no – longer influencing preference. It is expected that hunger state will influence motivation to consume food and thereby overcome neophobia to new foods more quickly. As birds are less hungry on days when fed AL than on days when they are fed QFR it is expected that they will be more likely to leave a food reward (or consume less of it) if presented on a day when they are fed AL than a day when fed QFR. This was observed in both this experiment and in experiment one. With the increase in exposure (number of training trials) to the food stuff this aversion is overcome and the food reward is fully consumed (as was observed). Thus, by the time of testing, the influence of neophobia may have been attenuated by experience.

Primarily as a consequence of the failure of the validation group to either fully consume the food reward or show a consistent preference for the food reward associated with the high deprivation (fed QFR on day of testing), it is impossible to meaningfully interpret the lack of preference observed in the QFR/CAP birds. Birds in this group fully consumed the food reward at all training points and failed to show an SDL preference in either direction. From these findings, it is not possible to ascertain the cause of this. Competing explanations include: firstly, the diet options may not have conferred differential levels of deprivation. Secondly, different quantities of the basal diet were consumed during training. Birds consumed slightly less basal diet when fed CAP than when fed QFR, which could have counteracted any satiating effects of CAP. Thirdly, a genuine lack of SDL – derived preference when food reward consumption is identical during training could explain the results. Finally, contrast effects may overshadow or cancel out any hunger – driven SDL preference. Here the bird experiences a large contrast between the daily food ration composition on days when fed CAP and given a food reward of starter crumb. On days when the bird is fed QFR the contrast is smaller. The differential contrast may lead to the birds developing preferences based on the contrast between diet option and food reward rather than on the basis of hunger state at time of training. However, in the absence of an adequate validation group, these comments remain speculative.

The quantity of food reward consumed during the five minute test differed according to the state of deprivation for QFR/AL birds. These birds consumed less food reward on days when they had been fed AL than on days when they were fed QFR, whereas, state during testing did not affect total food reward intake in QFR/CAP birds. It is tempting to conclude that this lack of a difference in the QFR/CAP birds is due to similar levels of deprivation. However, this would be misguided. D'Eath *et al.* (2009) highlighted that, when using short term feed intake rate to compare satiety levels in birds reared on either QFR or qualitative dietary restriction, which food to use in the test is problematic due to potential contrasts in quality. Although our birds had equal exposure to diets of both types, the presence of contrast effects may still be a problem. For example, if birds found the CAP diet less palatable than the QFR diet or the food reward (and informal observations suggested that they did) then they may have consumed more food reward on days when fed CAP due to the positive contrast in quality – even if they were more satiated when fed CAP than when fed QFR.

It is suggested that future studies should ensure that food rewards are sufficiently palatable to facilitate their full consumption during training irrespective of the state of deprivation. Further, studies using qualitative dietary restriction must ensure that the both diet options are

fully consumed on each day of training. Finally, studies should be modified to control for contrast effects to enable interactions between the two to be identified.

SDL Experiment 3

6.14. Introduction

There was no evidence from the previous study to suggest that CAP reduced the state of deprivation in the hungry broiler. However, it is possible that contrast effects cancelled out any effects of satiation in the CAP treatment group. Therefore, the final study attempted to control for contrast effects whilst using SDL to quantify the effects of a high fibre diet on feed – restricted broiler breeder welfare. Cellulose (Arbocel R®, J. Rettenmaier & Soene, Rosenburg, Germany) was used as a product that is readily available commercially and already in use in poultry production (to improve digestibility). Cellulose is an insoluble fibre and, thus, is poorly fermentable by avian gut microflora (Johnston *et al.,* 2003). However, it swells considerably when in contact with water (for example, Arbocel R® has a water binding capacity of 1:8, J Rettenmaier and Sohne, date unknown) and is expected to be associated with an increase in gut fill. Gastrointestinal signals signalling gut volume contribute to satiation (Powley and Phillips, 2004). Thus, cellulose may improve satiety in the feed – restricted broiler breeder and, thus, reduce the state of deprivation the bird experiences.

6.15. Hypotheses

It was hypothesised that broiler breeders would prefer a food reward previously associated with QFR over one previously associated with a diet quality – adjusted by the addition of cellulose (CEL) in a SDL task. Further, to identify whether contrast effects affect reward preference, some birds received a high energy density diet (same quality as the QFR ration) as their food reward and some birds received a low energy density diet (same quality as CEL) as their food reward. It was also predicted that birds would prefer the QFR diet to the CEL diet in a two – pan choice test. As before, a control group was also included (QFR/AL) to validate the methodology. To ensure that the cellulose – based diet resulted in the same growth rate as QFR a further group were reared on QFR (but did not take part in training and testing) to allow a growth curve comparison. Finally, the total food reward intake during the test periods was assessed. It was predicted that, if a difference was observed, QFR/AL birds would consume more when tested under high deprivation (fed QFR on that day) than under low deprivation (fed *ad libitum* on that day). Further, if cellulose reduced the state of deprivation by increased satiety, then irrespective of the composition of the food reward,

birds would consume more food reward when tested on the day that they had been fed QFR than on a day when they had been fed CEL. However, this was a minor hypothesis and the ability to detect this was reduced by the decision to reduce testing time back to three minutes per test (in case 'binge' feeding had affected SDL preference in experiment two).

6.16. Materials and methods

6.16.1. Subjects and treatment groups

This study used 40 Ross 308 broiler breeders obtained as day old chicks from a commercial hatchery (Aviagen, Stratford – upon – Avon, UK). They were group reared and spot – brooded according to producer recommendations for heating and temperature (Aviagen 2007). On day 29, birds were blocked according to weight and then allocated to one of four treatment groups. These were: 1) QFR/AL (n = 8), 2) QFR/CEL(QFR food reward) (n = 8), 3) QFR/CEL(CEL food reward) (n = 8) and 4) QFR (n = 16). The treatment variable was the diet protocol and the food reward that each bird received during the experimental phase.

6.16.2. Housing and husbandry

From day 29 birds were individually housed in mesh cages (0.6m L × 0.6m W × 0.8m H) in blocks of four cages (two cages back – to – back) with each adjacent cage housing a conspecific. The cage floor was covered with a wood tray filled with shavings to facilitate some natural behaviour. Water was available *ad libitum* and each bird was fed from a D – cup feeder located at the outermost corner of the cage. This was done to ensure that, whilst the other birds could see that a bird was feeding, they could not see what was being consumed, as this was essential to the study once the coloured food rewarded were introduced. Birds were weighed weekly throughout the experimental phase.

6.16.3. Feed and nutrition

All birds were fed at 09:00 and, from day 29, were fed according to their treatment group. Birds in the QFR/AL group alternated every two days between QFR and AL (*ad libitum* access to feed between 09:00h and 17:00h (days 29 – 48) or 09:00-14:30h (day 49 onwards). Birds in both the QFR/CEL(QFR) and QFR/CEL(CEL) groups alternated every two days between QFR and CEL. Birds in the QFR treatment were maintained on QFR throughout the study. Any food remaining at 17:00h (14:30h from day 49) was removed, weighed and intake recorded.

The basal diets consumed by all the birds was designed to meet the nutritional requirements of broiler breeders during the starter (day 1 – 35) and grower (day 36 onwards) phases (Target Feeds, Shropshire, UK). The starter mash contained 221 g CP / kg and 12.6 MJ ME / kg and the grower mash contained 211 g CP / kg and 13.2 MJ ME / kg. The CEL diet contained the same quantity of QFR with the addition of pure cellulose (Arbocel R®, J.

Rettenmaier & Soene, Rosenburg, Germany). The quantity of cellulose was gradually increased from 4% on day 29 to 10% on day 35 and was maintained at this inclusion rate throughout the phase of SDL training and testing.

The food rewards used in the SDL phase were either 15 g of basal ration (QFR/AL and QFR/CEL(QFR) birds) or 15 g of basal ration plus 1.7 g cellulose mixed in (i.e. similar inclusion rate to the CEL diet) (QFR/CEL(CEL) birds). The food rewards were either stained red or green using food colouring (Silverspoon, Cambridgeshire, UK) in a mix of one part food colouring to two parts water. To each 15 g portion of the basal diet was added 2.25 ml of the mixture. Due to the water-retaining properties of the cellulose, it was necessary to add 4.97 ml of the mixture to each 16.7 g portion. Diets were fed moist and not dried in a warm oven prior to use (unlike in the previous experiments). Water was added to try and improve palatability (see: Moritz *et al* 2001) and to ensure that the full food reward was consumed at each training session.

6.16.4. SDL protocol

SDL training and testing started on day 49 and finished on day 68. The SDL protocol was similar to the previous two experiments with the following exceptions. Firstly, the QFR birds did not take part in SDL training and testing. For birds involved in SDL training any food remaining was removed at 14:30 and replaced with a food reward. All birds were tested between 14:30 – 16:30h on the two days of testing.

Each SDL test lasted for three minutes, birds were offered 70 g each of the red and green food rewards and the test and data collection was conducted as previously described for experiments one and two.

6.16.5. Two-pan testing

On day 70 all birds in the QFR/CEL groups were given a "two – pan choice test" between the CEL diet and the QFR diet (70g available of each diet). This test took place between 14:30 – 16:30h. Birds were given three minutes of access to both diets (located side by side) before the diet options were removed, weighed and the intake of each diet was calculated.

6.16.6. Statistical analysis

All statistical analyses were carried out using the same tests described in experiment two.

6.17. Results

6.17.1. Bird growth rate as a function of treatment group

There were no significant differences between treatment groups in bodyweight at the start of the study (day 29; $F_{3,36}$= 47.0, P = 0.703). The mean (± SD) bodyweight of all birds at the start was 494.1 g (± 29.1 g). Although there was a significant effect of treatment on bodyweight on day 67 (end of study; $F_{3,36}$=335.36, P < 0.001; mean ± SD QFR/AL: 2318.6 ± 69.4 g; QFR: 1393.3 ± 85.3 g; QFR/CEL(QFR): 1406.3 ± 68.9 ; QFR/CEL(CEL): 1412.8 ± 49.0 g), examination of the least significant differences indicated that birds in the QFR/AL treatment were heavier than birds in the other three treatments, which did not differ from each other. This indicated that the birds' growth rate was not affected by the addition of cellulose to the dietary ration.

6.17.2. Daily food consumption during SDL training and testing

All birds, irrespective of treatment group, always consumed the full ration of QFR on all of the days that it was offered. To allow for growth over the course of the experiment, the quantity offered was increased in 2 g increments over the period of training and testing (from 43 – 49 g/day).

Birds in the QFR/CEL(QFR) and QFR/CEL(CEL) groups always consumed the full ration of the diet with added cellulose on days that this diet option was available. The quantity offered was increased in line with the increase in QFR. Therefore, the quantity of CEL consumed was (47.3 – 54 g/day).

Birds in the QFR/AL group consumed considerably more feed on days that they were fed *ad libitum*. The mean daily quantity (± SD) consumed over the duration of training and testing was 156.2 (± 15.2 g).

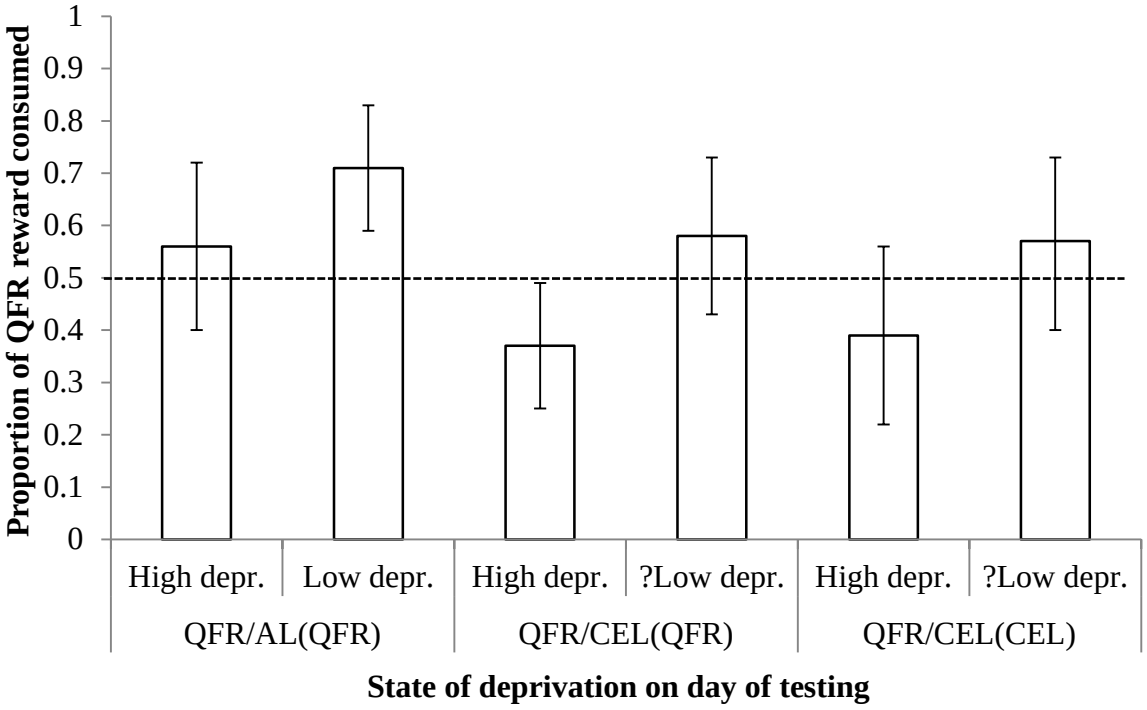
6.17.3. Food reward consumption on days of SDL training

In contrast to experiments one and two, all birds consumed the full food reward offered on each day of SDL training (irrespective of either treatment or diet option fed that day). Therefore, the birds had equal exposure to both the food reward associated with QFR and the food reward associated with the alternative diet option at the point of the first SDL test.

6.17.4. SDL preference test

No significant differences were found between the treatment groups ($F_{2,18.0}$=0.64, P = 0.537). There was an effect of diet fed on day of testing ($F_{1,18.0}$=7.66, P = 0.013), but no interaction between treatment group and diet fed on day of testing ($F_{2,18.0}$=0.06, P = 0.941) and no effect of test number (i.e. 1 or 2; $F_{1,18.0}$=1.8, P = 0.197). It appeared that birds consumed a greater proportion of the food reward associated with QFR when tested on days when they had not been fed QFR (see figure five). However, a post – hoc examination of the least significant differences (5% level) indicated this significant effect of diet fed on day of testing on reward consumption was not significant at the level of the treatment group and none of the treatment groups showed a preference for either food reward that significantly differed from the chance value of 0.5.

Figure 5 Experiment 3: The effect of treatment group and diet option fed (state of deprivation) on day of preference on QFR food reward consumed as a proportion of total feed consumed during the three minute food reward preference test. The error bars signify the standard error of the mean. A significant preference for either food reward was not observed for any treatment group. The prefix "?" is used to denote that, in contrast to the QFR/AL group, a state of relatively low deprivation when fed CAP is only an assumption for the birds in the QFR/CAP group.

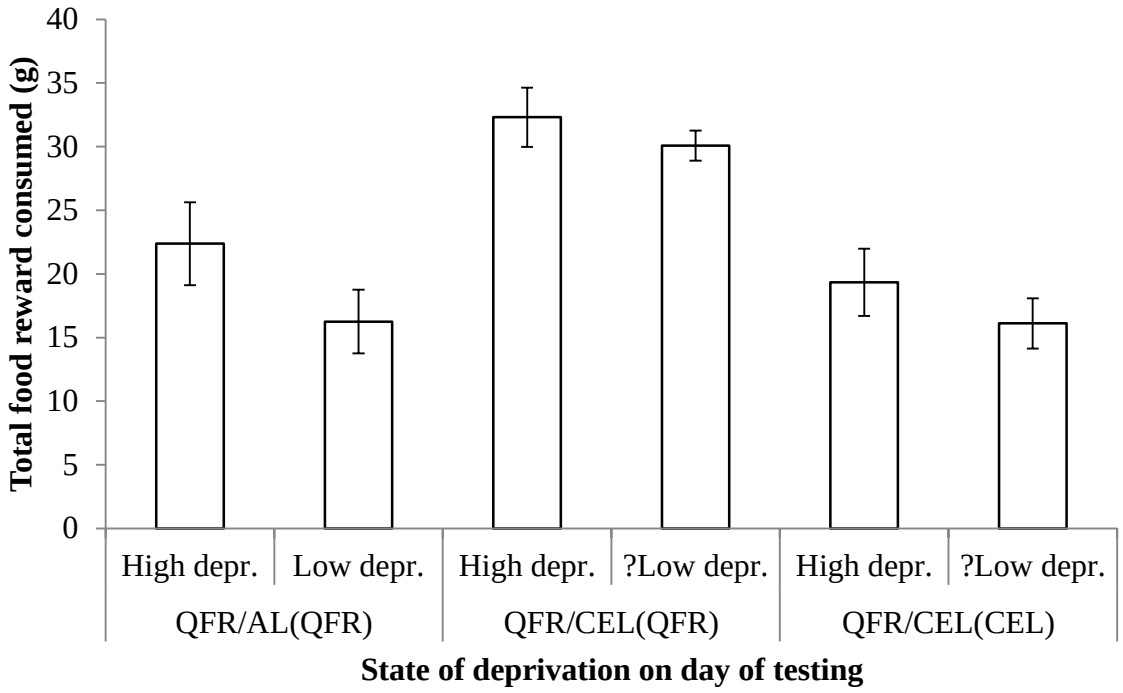


6.17.5. Total quantity of food consumed during each test

A significant effect of treatment group on total food reward intake during each test ($F_{1,18.01}$=22.06, P < 0.001) was observed. Post-hoc testing between groups using the least significant differences (5% level) indicated that birds in the QFR/CEL(QFR) treatment group consumed more food reward than birds in either the QFR/AL group or the QFR/CEL(CEL) group. There was no significant difference between the QFR/AL and QFR/CEL(CEL) groups.

Overall, birds consumed more during the SDL test when QFR was fed on the day of testing ($F_{1,22.4}$=5.26, P = 0.032) with no interaction between treatment group and diet fed on the day of testing ($F_{1,18.0}$=0.03, P = 0.859) (see figure 6). However, within treatment group, post – hoc testing using the least significant differences (5% level) did not identify any tendencies for food reward consumption to differ according to diet option fed on day of testing.

Figure 6: experiment 3: the effect of treatment and diet option fed on day of testing (state of deprivation) on total feed consumed during the three minute test. The error bars are the standard errors of the treatment means. The prefix "?" is used to denote that, in contrast to the QFR/AL group, a state of relatively low deprivation when fed CAP is only an assumption for the birds in the QFR/CAP group.



6.17.6. Two-pan diet preference test: cellulose versus QFR

Birds in the both the QFR/CEL(CEL) and QFR/CEL(QFR) groups showed a clear preference for the basal (QFR) diet over the CEL diet (T_{16}=16, P < 0.001) with 15 out of 16 birds only consuming the basal (QFR) diet and the remaining bird consuming less than 0.01 of the CEL diet as a proportion of total feed consumed during the three minute test. There was no effect of diet fed on the day of testing on the preference observed ($U_{7,9}$=27.0, P = 0.875).

6.17.7. Anecdotal observations

Unlike in the previous two experiments the birds did not observed any unexpected feeding behaviours such as exaggerated gaping or neck ripples.

6.18. Discussion

The lack of SDL preference observed in this experiment indicates that the apparent SDL preference observed in experiments one and two was probably an artefact of the test methodology that arose from differential consumption of the food rewards associated with high and low states of deprivation during training. However, Campbell *et al.* (1987) found a SDL preference (albeit for the low deprivation reward) in rats in which intake of rewards was matched during training under different states of food restriction. The actual reward may affect the SDL as when intake during training is matched, rats develop a SDL preference for sucrose but not saccharin solutions (Capaldi *et al.,* 1994). Further, SDL preferences towards the solution that was consumed in a lower quantity during training have been observed (Capaldi *et al.,* 1983). This indicates that quantity consumed during training may not necessarily be the cause of any preference observed during testing. This suggests that nutritious food rewards (such as the coloured starter crumb used in the current studies) support the development of an SDL preference even when feed intake during training is equal between food rewards. Consequently, it is not possible rule out differences in the quality of the food reward as affecting the development of an SDL.

The composition of the food reward has been found to affect rat preferences for a food reward associated with high deprivation. Rats showed an SDL preference when the food reward was unsweetened mash but no preference when the mash contained sweeteners (Capaldi *et al.,* 1991). The behaviour of the birds during testing in experiments one and two (exaggerated gaping and odd 'neck ripples' during swallowing) suggested that consuming the food was not pleasant (although an alternative explanation could be the dryness of the food made the food harder to swallow). These were not observed when feeding the moist food reward in experiment three. An increase in motivation to feed (under high deprivation) combined with a reduced perception of 'disgust' when consuming relatively unpalatable food could also explain the preferences observed for the food reward associated with high deprivation in experiments one and two but not three. Hunger – induced reduction in disgust at unpleasant food stuff has been documented in humans (Hoefling *et al.,* 2009) and it is possible that the food reward was perceived as less unpleasant under conditions of high deprivation. However, Campbell *et al*. (1987) performed a series of experiments aimed at identifying whether food reward linked SDL preferences formed due to an aversion for the less preferred reward or an increased attraction to the preferred reward. They concluded that

the conditioning phenomenon was caused not by increased aversion to one of the flavour rewards but by increased attraction to the other flavour reward. Although, it should be noted, that Campbell *et al.* (1987) did not use food rewards that were inherently unpleasant to consume whereas the behaviour of the birds in experiments one and two suggested that the food rewards were not particularly liked.

The finding that there was a diet – fed – on – day – of – testing dependent preference at the level of the experiment (experiment three) is more difficult to explain. State – at – time – of – testing dependent preferences have been observed in the literature both in the same direction as the current study (i.e. to prefer high deprivation rewards when tested under low deprivation, Revusky, 1967) and in the opposite direction (to prefer the high deprivation rewards when tested under conditions of high deprivation, Capaldi and Myers, 1982) so a genuine interaction cannot be ruled out. However, the fact that it was not present at the level of the treatment group and not observed in experiments one and two suggests that 'significant effect' of diet fed of day of testing on the bird's preference during testing was a chance finding rather than evidence of a genuine preference.

The findings related to the total food reward intake during the test deserve further consideration. It should be noted that the significant difference in total feed intake between QFR/CEL(QFR) and QFR/CEL(CEL) birds should not be taken to reflect increased hunger on the part of the birds rewarded with a basal diet food reward relative to those rewarded with the cellulose based food reward. The two – pan test demonstrated that the cellulose diet is less attractive than the basal (QFR) diet which may have contributed to a reduced intake during testing. However, the cellulose diet was also more voluminous and a similar quantity in volume consumed would be significantly different in terms of weight of food consumed. The lack of an interaction between state of deprivation at the time of testing and total food reward intake during testing indicated (in line with experiment one's findings) that three minutes is not long enough for state – driven differences in intake to be observed even in older birds in which the contrast in feed intake between QFR and AL was more severe. Visual assessment of the graph suggests a trend for food reward intake to be lower on days when birds were fed AL or CEL. Whilst no conclusions can be drawn with these results it would be interesting to study this further with a longer test period as the pattern appeared independent of any potential contrast effect.

Finally, the two – pan test conducted at the end of the study clearly indicated that the birds preferred the basal (QFR) diet to the CEL diet. These results do not demonstrate whether this was primarily due to an aversion to CEL, a preference for the basal diet or a combination of

both factors. Despite this, informal observations indicated that the birds did not like the CEL diet, showed increased vocalisations on being fed this diet and showed an increased latency to start eating it (not formally measured). Interestingly, this was not witnessed with the coloured moist CEL – based food reward suggesting that it was the texture / dryness of the cellulose diet that was unpleasant rather than the cellulose per se. This preference for the high energy density diet mirrors previous findings by both the authors (Buckley *et al.,* 2011a) and others (e.g. see: Drewnowski, 1998; Day *et al.,* 1999; Guillemet *et al.,* 2007). Here, this preference for the basal diet over the CEL diet occurred following several weeks' exposure to both diets to facilitate the association of each diet with its post – ingestive effects but a preference for the high energy dense diet was still observed. The preference of a feed restricted animal for a high energy diet when offered *ad libitum* could be a feeding strategy (Guillemet *et al.,* 2007). In other words, the preference could be circumstantial rather than an absolute preference. Whilst this cannot be ruled out, the behaviour of the birds towards each diet option when fed on the alternating diet schedule, suggests that the birds genuinely did not like the CEL diet. This should be considered further in any future preference tests evaluating QFR and qualitative dietary restriction regimes even if qualitatively restricted diets are shown to enhance satiety and thus reduce the state of deprivation the bird experiences. Good welfare is about removal of unpleasant stimuli *and* the provision of positive stimuli (Yeates and Main, 2008).

6.19. Overall discussion and conclusions

Overall, these studies do not provide strong support for the further use of SDL to quantity the satiating effects of QFR or qualitative dietary restriction. However, they do not completely rule out its use either as there are still some ambiguities surrounding the findings – both in relation to each other and to other studies that have been published in this area. Therefore, it is pertinent to conclude this chapter with some discussion regarding both the methodology adopted in the current studies and also to explore some of the contradictions in the published studies in SDL and implications for future work in this area.

A number of issues are relevant to any discussion of methodology. These include sample size, number of trials, the use of coloured food rewards and the use of cues which were concurrent with the reward itself. The sample sizes (per treatment group) used in the current study were four (experiment one), twelve (experiment two) and eight (experiment three). Although experiment one was unexpectedly smaller, the sample sizes used in experiments two and three are similar to sample sizes that yielded significant results for other authors (e.g

.n = 4, Vasconcelos and Urcuioli, 2008; n = 6, Pompilio and Kacelnik, 2005; n = 8, Clement *et al.*, 2000; n = 12, Kacelnik and Marsh, 2002; Friedrich and Zentall, 2004; Marsh *et al.*, 2004) although less than several others (e.g. n = 13, Aw *et al.*, 2009; n = 16, Gipson *et al.*, 2009). The number of stimulus / food reward – deprivation state pairs was four per state prior to the first test and eight by the second test in the current study. This is much less than the number of trials that it took for animals to learn to associate a distal cue with a food reward under two different states (e.g. 40 trials, Marsh *et al.*, 2004; 120 trials, Vasconcelos and Urcuioli, 2008). However, using a methodology in which the flavour of the food was the cue, Capaldi *et al.*, (1983) observed a SDL after three flavour – deprivation pairings. This suggests that tasks where the cue is part of the food reward are easier to learn than cues that are more distal to the food reward.

The current experiments used identical food rewards stained different colours with food colouring. Although chicks show innate preferences (or aversions) for food stained different colours (Roper and Marples, 1997), these preferences are modified where the outcome of feed consumption is rewarded (Kutlu and Forbes, 1993). No significant preferences for colour or interactions with other effects were observed in this study suggesting that colour biases were not a problem.

Coloured food rewards were used to more closely link the stimulus that cued food reward with the act of consuming the food. Previous studies by the authors suggested that hungry broiler breeders found distal cues (e.g. Y – maze colour arms) to signify differences in food quality or quantity difficult to learn (Buckley *et al.*, 2011a, 2011b). Further, maze methods such as those utilised by Kurtz and Jarka (1968), Pompilio *et al.* (2006) and Aw *et al.* (2009) would be problematic to interpret as deprivation state has been shown to enhance side biases (Talling *et al.*, 2002) and was a serious impediment in previous studies by the authors (Buckley *et al.*, 2011a, unpublished observations). Thus, the experiments in this chapter aimed at the opposite approach: coupling stimulus with reward to maximise associative strength. However, this may have affected SDL development. Most of the previous studies that observed a SDL preference for the stimulus associated with high deprivation used cues distal to the food reward. In other words, the cues used were linked in some way to appetitive behaviour rather than consummatory behaviour. For example, pecking keys (Friedrich and Zentall, 2004; Marsh *et al.*, 2004; Pompilio and Kacelnik, 2005; Gipson *et al.*, 2009), positional cues (Kurtz and Jarka, 1968; Aw *et al.*, 2009), colour cues (maze arms) (Aw *et al.*, 2009) or distance flown (Kacelnik and Marsh, 2002. SDL preferences for the reward associated with high deprivation have been identified when the cue is more closely linked

with the food reward (e.g. scent, Pompilio *et al.*, 2006; flavour, Capaldi *et al.*, 1991; Capaldi *et al.*, 1994). However, the opposite effect has also been observed with animals showing a preference for the reward associated with low deprivation (e.g. flavour, Capaldi and Myers, 1982, Capaldi *et al.*, 1983) or no effect at all (e.g. flavour and artificial sweetners, Capaldi *et al.*, 1994). This suggests that the phenomenon of SDL is neither as robust and the predicted direction of effect as clear cut when using rewards in which the conditioned stimulus associated with state of deprivation is the actual reward consumed.

The timing of the food rewards (closeness in proximity to daily feeding) (Capaldi and Myers, 1982) has been shown to affect the direction of the SDL preference. Food rewards given just before and after feeding result in conditioned preferences for the high deprivation food reward (Revuksy, 1967). By contrast, food rewards given at a longer time interval from the start and finish of daily food consumption condition a preference for the low deprivation food reward (Capaldi and Myers, 1982; Capaldi *et al.*, 1983) although not always (Capaldi *et al.*, 1991). In our experiments the low deprivation reward was given directly after AL (but not CAP or CEL where latency to consume the full daily ration showed considerable variation between individual birds, see: chapter 5). However, the high deprivation food reward was temporally separated from the preceding daily meal by 6 – 7 hours and the succeeding one by 15 hours. This may have affected the development of SDL preferences. Further, both the presentation and the quantity of the food reward demonstrably affect SDL preference presence and direction (Capaldi *et al.*, 1991). Rats given unsweetened mash show a preference for the reward associated with high deprivation regardless of quantity of reward offered (1 g or 16 g per training session). However, sweetening the mash with saccharin inhibited SDL preferences when the food reward was 16 g but not 1 g and increasing this artificial sweetness further inhibited SDL preferences with the smaller reward also. Further research is needed to quantify the effect of deprivation state on preference for different macronutrients and energy density of food rewards to further understand the effect of deprivation state on SDL preference development (Capaldi *et al.*, 1991) as these are likely to interact to influence both the development and direction of any SDL preference.

6.20. Conclusions and recommendations for future research

In conclusion, there was no evidence of an SDL preference when intake of food rewards associated with high and low deprivation were matched during training (experiment three). However, the literature suggests both the development and direction of SDL preferences is complex, nutrient and context specific when using a methodology in which the cue linked to

deprivation state is also identical with the food reward. This additional complexity may limit the value of SDL methodologies similar to the one used here. Further research should concentrate on using distal, appetitive cues, and include more training trials prior to testing. Finally, the evident lack of preference for a high fibre diet when offered a free choice (in a two – pan test) suggests that the sensory aspects of food preference should receive greater consideration when quantifying the welfare effects of QFR and qualitative dietary restriction.

6.21. Acknowledgements

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7. Overall summary and recommendations for further research

7.1. Summary of this programme of research

This programme of research aimed to identify broiler breeder dietary preferences for either qualitative or quantitative dietary restriction. It is concluded that there is no evidence that hungry broilers or broiler breeders want or prefer a diet that is quality – adjusted by the addition of calcium propionate, oat – hulls or cellulose. Thus, the findings of this thesis do not indicate that qualitative dietary restriction confers welfare benefits. Where preferences where expressed these were for the conventional high energy density diet. However, despite this, the conditions under which the birds predominantly expressed a preference were predominantly those where the feed restricted broiler breeder had been released from feed restriction and allowed to consume both diets *ad libitum* during the test situation. However, this is an artificial situation far removed from the broiler breeder living within the commercial situation and may not reflect the state of the bird when exclusively fed one of the diets Notably, the presence of hunger – state dependent effects both on the ability to learn or express a preference complicates the interpretation of the findings and attempts to utilise the presence of these affects as an experimental variable rather than as a nuisance variable did not prove successful in final experiment(s) (chapter six).

7.2. A cautionary note on the use of quality – adjusted diets to improve welfare

At the inception of this programme of research the primary focus of thought was on whether a qualitatively – adjusted diet would reduce hunger (a negative affective state) and improve feelings of satiety (a positive affective state). Thus, it was largely assumed that qualitative dietary restriction would either be a benign intervention that had no effect (i.e. would not reduce hunger state) or a positive effect (i.e. would reduce hunger state). Whilst the possibility that calcium propionate might have detrimental effects was acknowledged this was not extended to include the fibrous components – oathulls and cellulose – that were to be added to the diet. This assumption seems to be prevalent across the scientific literature. No papers were found which highlighted the possibility that the birds may simply not find qualitatively dietary restriction palatable and that their welfare would be negatively affected by any dislike of the diet composition. However, this may have been erroneous. The possibility that feeding a diet that is less liked by the birds, and that has no effect (or very

little or not a lasting effect) on satiety may actually remove what may be one of the few very rewarding / pleasurable experiences in the commercial broiler breeders' existence, should be acknowledged. It is increasingly recognised (e.g. Yeates and Main, 2008; Balcombe, 2009) that good animal welfare is about promotion of positive affective states (e.g. pleasure) and not just the removal of negative affective states. Thus, if the birds demonstrably do not like qualitative dietary restriction, regardless of its satiating (or otherwise) effects, then this should be factored into any decision – making regarding the diet regime that the birds should be offered.

This programme of research was very hands on and the author was the primary researcher but also the primary stock person / carer of all the birds used in these experiments (and other, incomplete, trials that were not included in this thesis). Thus, the author had a wealth of opportunity to informally observe the birds throughout the three years of experimental work. Anecdotal observations of the birds indicated that they did not like the qualitatively restricted diets. Relevant observations included some of the birds trying to escape their pens when offered the calcium propionate diet. They also showed an increase in appetitive feeding behaviour (more sifting through the feed with their beaks and / or foot scratching in the wood shavings) and transient episodes of feed sampling (rather than consuming a meal) that were interspersed with appetitive behaviour. Quiet vocalising at the point at which new feed was offered if the feed was qualitatively restricted which was also coupled with the other signs described.

Appetitive feeding behaviours are identifiable part of the natural feeding behaviour of jungle fowl (the progenitor species, see: Dawkins, 1989). Thus qualitatively restricted diets are claimed to improve the birds' welfare by enhancing the ability of the bird to perform more naturalistic feeding behaviour (Kyriazakis and Tolkamp, 2011). However, it is argued here that this is unlikely to be the case for broiler breeders. Performing appetite behaviour such as foot scratching whilst in the presence of food is effectively paying an un – necessary additional cost to obtain food. Hunger is known to reduce contra – freeloading in chickens (Lindqvist *et al.,* 2002) but this begs the question: are the qualitatively restricted birds showing an increase in this 'un – necessary additional cost' because they are less hungry (so more motivated by other factors e.g. information gain, Lindqvist *et al.,* 2002)? Or, do they because the feed quality is considered to be so poor that the birds are motivated to seek an alternative source of food?

Various informal observations in relation to the birds feeding behaviour when fed the high energy density diet support the argument that the increase in appetitive behaviour observed

may represent a decrease in the birds' satisfaction with the diet offered. It was noted that birds fed *ad libitum* or in the eighty % treatment group (experiment two, chapter four) showed very little appetite behaviour either during feeding or between feeding bouts. In contrast, birds in the forty % and control (fed to commercial levels of feed restriction) groups did but appeared to *only* once the entire daily feed ration had been consumed. This suggests that the performance of foot scratching / beak raking was not a fixed behavioural pattern triggered by the presence of food and necessarily tightly coupled to consummatory behaviour (feeding). Rather, it was performed as a consequence of a frustrated motivation to feed. Further, in a preliminary trial (not reported in this thesis), birds that had problems accessing the feeder showed a similar shift towards increased appetitive behaviour. Birds were observed to excavate through the wood shavings to expose large areas of concrete flooring interspersed with small episodes of actual feeding from the feeder (which was slightly too high for comfortable feeding). This was not observed when the birds were able to access their feed more easily. Thus, it is proposed that an increase in appetitive foot scratching behaviour or a change in the sequencing of behaviours in the presence of feed could potentially be used as a marker of broiler breeder satisfaction with feed quality.

There is a body of evidence (see review by Rodgers *et al.,* 2010) that suggests that changes in sequences of behaviour can be used to assess the effects of diet quality / composition or the administration of drugs known to affect appetite on the animals' satiety. Formally, known as 'behavioural satiety sequencing' it relies on the fact that animals (predominantly rats) have been shown to follow predictable patterns in behaviour. In these predictable patterns the animal eats to a point of satiety (assumed) when fed the 'normal diet', then tends to spend a period grooming, followed by a period of rest (Rodgers *et al.,* 2010). However, the adulteration of diets with compounds known to be unpalatable such as simmondsin (e.g. Lievens *et al.,* 2009) and quinine (e.g. Ishii *et al.,* 2003) disrupts this sequence of behavioural events; whereas, a caloric preload or the administration of natural physiological compounds (e.g. cholecystokinin) known to be part of the satiation process does not (Halford *et al.,* 1998). Thus, the application of 'behavioural satiety sequence' methodologies may be a promising route of exploration to examine the reasons for the efficacy of qualitative dietary restriction at reducing energy intake in broiler breeders.

7.3. Suggestions for further research using indirect measures of state or preference

Despite the failure of this programme of research to resolve the conundrum of whether qualitative feed restriction improves the welfare of feed restricted broiler breeders it is imperative that the search goes on to quantify this. Further research should focus on developing methodologies that exploit the effects of different dietary regimes on the broiler breeder cognitive and emotional state in order to indirectly use the choices that the bird makes in order to assess its welfare state. An example would be to assess state – dependent learning using an operant set up in which the conditioned stimulus is not a food reward but a defined appetitive response that provides access to a food reward (similar that utilised by Marsh *et al.,* 2004). Alternatively, methods could be devised that do not involve the animal alternating between two diet options but, instead, examine the effects of being reared exclusively on a given diet (i.e. either quantitative or qualitative feed restriction or *ad libitum* access to commercial feed) on an animal's choices. For example, behavioural satiety sequencing (discussed in section 7.4.) could be used.

Judgement biases have been used to assess other welfare situations and show promise as a wider tool for quantifying animal welfare (Burman *et al.,* 2009) Whilst in the animal literature this has largely focussed on affective biases (e.g. Harding *et al.,* 2004; Bateson and Matheson, 2007; Sanger *et al.,* 2011) the human literature indicates that other biases may be valuable in the quantification of hunger state. Humans have been observed to bias attention towards food related cues when tested under conditions of acute feed restriction (Frank *et al.,* 2010; Piech *et al.,* 2010; Tapper *et al.,* 2010) and this could prove a more promising approach to hunger quantification in broiler breeders.

Alternatively the effects of feed restriction *per se* and type of feed restriction on the foraging decisions of the broiler breeder could be investigated. Risk sensitivity is a foraging phenomenon that has yet to be explored as a welfare tool but could show promise as a tool for identification of severe hunger states. Animals are normally risk averse but sometimes become risk prone when in a state of energy deficit (see: Kacelnik and Bateson, 1996; Bateson and Kacelnik, 1998) which, it is presumed is associated with a feeling of chronic hunger. It is thus proposed that this could be used to produce a curve at which a broiler breeder moves from being risk aversive to indifferent to risk prone by using birds reared to different degrees of quantitative feed restriction. The effect of different types of qualitative feed restriction could then be plotted against this curve to assess the severity of feed restriction.

Finally, feed restriction is known to alter how an animal responds behaviourally to a test situation. Side biases are more common in feed restricted animals (e.g. Pigs, Van Rooijen and Metz, 1987; Starlings, Talling *et al.*, 2002; Broiler breeders, Buckley *et al.*, 2011a, 2011b) and so differences in side bias severity when learning either a food - or non – food rewarded task could be used to compare different diets. However, for all of these proposals there is a need for some good quality preliminary studies to ascertain the effects of feed restriction *per se* on the various cognitive and emotional processes and the resulting behavioural outputs.

Despite the difficulties facing researchers trying to quantify broiler breeder hunger and diet preference, feed restriction remains the most critical welfare issue facing the modern day, fast growing, broiler breeder. Thus, it should remain of key interest and a priority for welfare scientists, particularly given the continued and increasing popularity of poultry meat and the demand for this meat to be readily available and affordable.

7.4. References

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